POSTPRANDIAL EFFECTS OF THREE ISOCALORIC HIGH-FATMEALS WITH DIFFERING LIPID LOADS ON TRIGLYCERIDES, OXIDATIVE STRESS, AND ENDOTHELIAL FUNCTION

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POSTPRANDIAL EFFECTS OF THREE ISOCALORIC HIGH-FAT MEALS WITH DIFFERING LIPID LOADS ON TRIGLYERCIDES, OXIDATIVE STRESS, AND ENDOTHELIAL FUNCTION

BACKGROUND: There have been numerous studies that compare the relationship of postprandial lipemia, oxidative stress, and endothelial dysfunction, but there is a lack of information as to the dose response nature of isocaloric high-fat meals (HFM). **OBJECTIVE**: To examine the dose response of lipemia (isocaloric HFM consisting of ~25%, ~50%, and ~75% fat) on plasma triglycerides (TG), oxidative stress, and endothelial function. It was hypothesized that the highest fat load would produce the greatest amount of oxidative stress and endothelial dysfunction; whereas each lipid load would be significantly higher than the previous. **METHODS**: Ten young inactive healthy men (22.8 ± 2.9 yrs) participated in three randomized challenge meals consisting of 25%, 50%, and 75% fat. Endothelial function, as measured by flow-mediated dilation (FMD) and blood samples were taken at baseline, 2 and 4 hours postprandial. Samples were assayed for blood biomarkers of TG and oxidative stress (3-nitrotyrosine (3-NT) and thiobarbuiuric acid reactive substances (TBARS)). **RESULTS**: TG were found to be significant with the 50% fat meal compared to the 25% fat meal ($p = .001$); but not between the other comparisons. Significance was also found for TG between 25% and 50% fat meals at 2 hours postprandial ($p = .000$) but not for any of the other comparisons. No changes were observed with either measure of oxidative stress. FMDs were found to be significant with the 50% fat meals compared to the 25% fat meal ($p = .026$), and the 75% fat meals compared to the 25% fat meal ($p = .002$); but not between the 50% and 75% fat meals ($p = .142$). Significance was also found for FMDs at 2 hours postprandial between 25% and 75% fat meals ($p = .027$) and at 4 hours postprandial between 25% and 50% ($p = .017$) and 25% and 75% fat meals ($p = .013$). **CONCLUSIONS**: Thus, it appears young healthy inactive men do not exhibit a dose response in lipemia following an isocaloric HFM consisting of 25%, 50%, and 75% fat. Interpretation of the oxidative stress and endothelial dysfunction results are more difficult to interpret without a dose response in lipemia. However, other measures of oxidative stress should be considered before strong conclusions can be drawn.

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Article for The American Journal of Clinical Nutrition

Postprandial effects of three isocaloric high-fat meals with differing lipid loads on triglycerides, oxidative stress, and endothelial function

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Abbreviations: CVD, cardiovascular disease; FFA, free-fatty acid; FFQ, food frequency questionnaire; FMD, flow-mediated dilation; HFM, high-fat meal; MDA, malodialdehyde; NO, nitric oxide; PA, physical activity; TBARS, thiobarbuiuric acid reactive substances; TG, triglycerides; 3-NT, 3-nitrotyrosine.

Abstract

BACKGROUND: There have been numerous studies that compare the relationship of postprandial lipemia, oxidative stress, and endothelial dysfunction, but there is a lack of information as to the dose response nature of isocaloric high-fat meals (HFM). **OBJECTIVE**: To examine the dose response of lipemia (isocaloric HFM consisting of ~25%, ~50%, and ~75% fat) on plasma triglycerides (TG), oxidative stress, and endothelial function. It was hypothesized that the highest fat load would produce the greatest amount of oxidative stress and endothelial dysfunction; whereas each lipid load would be significantly higher than the previous. **METHODS**: Ten young inactive healthy men (22.8 ± 2.9 yrs) participated in three randomized challenge meals consisting of 25%, 50%, and 75% fat. Endothelial function, as measured by flow-mediated dilation (FMD) and blood samples were taken at baseline, 2 and 4 hours postprandial. Samples were assayed for blood biomarkers of TG and oxidative stress (3-nitrotyrosine (3-NT) and thiobarbuiuric acid reactive substances (TBARS)). **RESULTS**: TG were found to be significant with the 50% fat meal compared to the 25% fat meal ($p = .001$); but not between the other comparisons. Significance was also found for TG between 25% and 50% fat meals at 2 hours postprandial ($p = .000$) but not for any of the other comparisons. No changes were observed with either measure of oxidative stress. FMDs were found to be significant with the 50% fat meals compared to the 25% fat meal ($p = .026$), and the 75% fat meals compared to the 25% fat meal ($p = .002$); but not between the 50% and 75% fat meals ($p = .142$). Significance was also found for FMDs at 2 hours postprandial between 25% and 75% fat meals ($p = .027$) and at 4 hours postprandial between 25% and 50% ($p = .017$) and 25% and 75% fat meals ($p = .013$). **CONCLUSIONS**: Thus, it appears young healthy inactive men do not exhibit a dose response in lipemia following an isocaloric HFM consisting of 25%, 50%, and 75% fat. Interpretation of the oxidative stress and endothelial dysfunction results are more difficult to interpret without a dose response in lipemia. However, other measures of oxidative stress should be considered before strong conclusions can be drawn.

Introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of morbidity and mortality in western society and will soon become the pre-eminent health problem worldwide [1, 2]. Atherosclerosis originates in the inner most cellular lining of the artery, the endothelium. The endothelium is a key regulator of vascular homeostasis, due to it not only functioning as a barrier for the vessel but through anti-atherogenic functions [3]. The endothelium acts to maintain the balance between vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell proliferation and migration, thrombogenesis and fibrinolysis [4, 5]. Certain circumstances that occur as a natural part of aging or additional perturbation (i.e. oxidative stress from consumption of a high-fat meal) can disturb the balance compromising the protective functions of the endothelium. The impairment of this vascular endothelium, or endothelial dysfunction leads to CVD [6-9].

It is well established that a high-fat meal (HFM) is a direct source of oxidative stress [10] and postprandial lipemia may represent an independent risk factor for atherosclerotic CVD. [11] The influx of free fatty acids (FFA) after a HFM leads to advanced oxidative stress, causing inhibition of nitric oxide (NO) production and bioavailability, which compromises the protection of the vasculature. Thus, postprandial oxidative stress from a HFM is proposed to be the source of endothelial impairment [11].

Correlations exist to support this connection [12]. More advanced research regarding a dose response among lipemia, oxidative stress and endothelial dysfunction is limited. There have been numerous studies that have compared the relationship between postprandial lipemia, oxidative stress, and endothelial function following a high-fat meal [6, 12-18], low-fat and high-fat meals [19-21], and even the effect of different lipid loads on triglycerides (TG) [22-24] and biomarkers of oxidative stress [24] . Tsai *et al.* [18] compared postprandial lipemia, oxidative stress, and endothelial function after a single meal consisting of 50% fat and found that triglycerides (TG) and oxidative stress increased at 2 and 4 hours postprandial, while endothelial function, as measured by flow-mediated dilation (FMD), decreased during the same time point. This demonstrates the correlations between lipemia, oxidative stress, and endothelial function but with only a single HFM. Cohen *et al.* [22] compared the effect of three HFMs consisting of 100 mL, 200 mL, and 300 mL of cream on TG response and found that TG response was different between each of the meals, with the meal consisting of 300 mL of cream having the largest TG response, and the peak to occur 2 hours postprandial.

Bloomer *et al.* [24]demonstrated a difference in TG and oxidative stress with two100% fat meals consisting of 33 g and 66 g of cream, with both TG and oxidative stress being higher in the 66 g meal compared to the 33 g meal, but did not examine endothelial function. In order to investigate the relationship between lipemia, oxidative stress, and endothelial function, the dose response nature of isocaloric HFMs should be examined. Therefore, the purpose of this study was to investigate the dose response of three isocaloric HFM consisting of ~25%, ~50%, and ~75% fat on plasma TG, blood biomarkers of oxidative stress, and endothelial function. It was hypothesized that the highest fat load would produce the greatest amount of oxidative stress and endothelial dysfunction; whereas each lipid load would be significantly higher in TG, oxidative stress, and endothelial dysfunction than the previous.

Methods

Research Design

The study was conducted in a randomized repeated measure design. Subjects consumed three separate meals consisting of 25%, 50%, and 75%, over a 7-14 day period, with at least 1-2 days between meals. Blood samples and brachial artery flow-mediated dilation (FMD) were performed before each meal and at 2:00 and 4:00 hours in the postprandial period. Blood samples were analyzed for plasma TG and biomarkers of oxidative stress, as measured by specifically 3-nitrotyrosine (3-NT) and thiobarbituric acid reactive substances (TBARS). The study design was approved by the University institutional review board.

Subject Selection

Ten young, healthy men were recruited, reviewed and signed the informed consent, and completed the study. All subjects participated in minimal physical activity (PA) (< 90 min/week) based upon previous research [25, 26]. The criterion for minimal PA was chosen to be less than the Surgeon General recommendation for individuals to participate in at least 150 minutes of physical activity weekly. All subject met the following criteria: were not lactose intolerant, no existing coronary artery disease, no existing diabetes, no existing pulmonary disease, not currently taking any vaso-active medications that might interfere with FMD measurements, not currently taking any cholesterol lowering medication (i.e. statins), normal cholesterol (<240

mg/dL) and/or triglycerides (<200 mg/dL),and no existing gallbladder disease. Subject descriptive demographic data are seen in Table 1.

Study Procedure

Each subject completed an initial screening phase before completing the three randomized high-fat challenge meals.

i. Screening Phase

The screening phase included a fasting blood draw, laboratory testing, completing a Medical History-Heath Habit Questionnaire, and completing a (3 month) Food Frequency Questionnaire (FFQ) (MSEL, Hutchinson Cancer Research Center, Nutrition Assessment Shared Resource, Seattle, WA). All subject completed the fasting blood draw at the IU Health Center to analyze for lipid profile. Height (cm), weight (kg), BMI (kg/m^2) and waist circumference (cm) were collected for each subject. Waist circumference was measured using an inelastic vinyl tape measure (Creative Health Products, Ann Arbor, MI). The site for the waist was the horizontal plane, at the level of the narrowest part of the torso, between the 10^{th} rib and the iliac crest; with the subject standing erect, with relaxed abdomen, arms by the side, and feet together. Three measurements were taken to the nearest 0.1 cm; the average of the three measurements was used to calculate the waist circumference. Variables of interest from the FFQ were energy intake (kcal/day); nutrient intake (total fat & saturated fat, carbohydrate & protein; g or percent of total caloric intake), and dietary antioxidants (E; IU & C; mg).

ii. High-Fat Meal Challenge

Subjects reported to the Clinical Exercise Physiology lab on three separate occasions and remained in the lab for 5-6 hours during each testing session. All subjects were instructed to fast for 12 hours and abstain from caffeine, vitamin supplements (including any antioxidant), and tobacco for 12 hours before reporting to the Clinical Exercise Physiology Laboratory. In addition, each subject was asked to abstain from physical activity/exercise 24 hours prior to the challenge meal. The meal the night prior to testing was not controlled.

The procedures for the high-fat meal challenge, brachial artery FMD, and repetitive blood draws are outlined below.

a. Challenge Meals

The high-fat meal was given between 6:00-9:00 am, depending on the subject's schedule. All three meals were given at the same time for each subject. The composition of the three isocaloric meals are summarized in Table 2 and consisted of a mixture of Ensure®, Ensure Plus®, and heavy whipping cream and had a fat content of 25%, 50%, or 75%. The order of the meals was randomized for each subject. The subject was instructed to not eat anything except for the test meal during testing. Water was allowed ad libidum.

b. Brachial Artery FMD Procedure

Brachial artery FMD was measured as previously described [27]. Subjects underwent an acclimatization phase (20 min) in order to obtain hemodynamic steady state by lying supine in a dark, climate controlled room (22-24^{o}C), with their arms extended laterally. A Hokanson brachial artery cuff (Hokanson, Bellevue WA) was placed on the subject's forearm to elicit brachial artery occlusion. The ultrasound image of the brachial artery was obtained longitudinally 2-10 cm above the antecubital fossa by 2D high resolution Terrason t3000 (Teratech Corporation, Burlington, MA) ultrasound system, using a 7 MHz linear transducer. Baseline brachial artery diameter and Doppler flow images were continuously recorded for 10 cardiac cycles (approx. 30 sec). Following baseline measurements, forearm occlusion will be elicited and maintained for 5 minutes by inflating the cuff to 250 mmHg. After the 5 minute occlusion, the cuff was released and brachial artery diameter and Doppler flow images will be continuously recorded for an additional 3 minutes. The arterial diameters and blood flow velocity were identified and measured using the Vascular Analysis Integrative System and software (Medical Imaging Applications, Coralville, Iowa). The baseline artery diameter was compared to the maximal diameter found post-occlusion, in order to determine % change in dilation. The equation for calculating percent change in FMD is as follows: ((peak hyperemic diameter – baseline diameter) / (baseline diameter)) *100.

c. Repetitive Blood Draws

Three blood draws were collected; baseline, and 2:00 and 4:00 hours post-meal. For the purpose of collecting plasma samples, IV access was obtained in the non-FMD arm or the back of the hand with a 22g oangiocath equipped with a PRN adapter and maintained for the duration of the treatment period. The IV access was flushed with normal saline (Hospira Pharmaceruticals). Venous blood samples (10-20 ml) were collected through the IV access and into ethylenediaminetetraacetic (EDTA) Vacutainer tubes (Vacutainer, Becton and Dickinson, Meylan, France) and separated by centrifugation within 30 minutes. Plasma was stored in 1.0 ml aliquots at -80^{o}C until analysis.

The plasma samples were analyzed for blood biomarkers of oxidative stress, 3-NT and TBARS, and TG, using commercial assay kits. Standard curves for all assays were developed in order to determine the concentrations in the study samples. 3-NT was analyzed in plasma using a 3-Nitrotyrosine ELISA kit as described by the manufacture (Abcam, Cambridge, MA; ab116691). TBARS was analyzed in plasma using a commercial assay kit according to manufacture specification by determining the concentration of malodialdehyde (MDA) (Cayman Chemical, Ann Arbor, MI). TG was analyzed in plasma using a commercially available colorimetric kit (Cayman Chemical, Ann Arbor, MI). All assays were performed in duplicate on first thaw of the samples after being stored at -80^{o}C.

Statistical Analysis

Descriptive statistics were used to analyze demographic data. Planned comparisons t-test were conducted in order to determine if there were differences in dependent variables among the meals of 25%, 50%, and 75% fat. One-way repeated measure ANOVA was performed to analyze simple main effects between meals during measurements at baseline, 2 hours and 4 hours postprandial. When ANOVA was used to test a hypothesis, Tukey HSD was applied for follow-up to a significant F-ratio. Alpha level was set at $p < 0.05$ for a two-tailed comparison. All statistical calculations were performed using SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Subjects

Complete data was collected on all 10 subjects, with the exception of blood data from two subjects at two separate time points. All subject reported participating in < 90 minutes of weekly physical activity, with most of their activity consisting of walking. The dietary data for all subjects during the three months prior to testing is shown in Table 3. The dietary intake for all subjects was consistent with current dietary recommendations of consuming approximately 2000 kcal/day, with 30-35% of those calories coming from fats. The recommended daily allowances (RDA) for vitamin E and C for men are 22.5 IU and 90 mg/day, respectively. No subjects were taking any vitamin supplements.

Triglycerides

TG for the three challenge meals is presented in Figure 3. TG were found to have a significantly higher concentrations with the 50% fat meal compared to the 25% fat meal ($p = .001$); but not between the other comparisons. A simple main effect was found between 25% and 50% fat meals at 2 hours postprandial ($p = .000$) but not for any of the other comparisons.

Oxidative Stress

Oxidative stress over the three challenge meals, as measured via TBARS assay, is presented in Figure 2. There were no statistically significant differences measured among any of the meals or time points ($p > .05$). Detectable 3-NT concentrations were found in only four out of the 10 subjects; therefore data was not analyzed or illustrated.

Flow Mediated Dilation

The baseline brachial artery diameters for the three challenge meals are displayed in Table 4. There were no significant differences in diameters among any of the meals or time points ($p > .05$).

Data for pre- and postprandial FMD for the three challenge meals are presented in Figure 3. FMDs were found to have significantly lower values with the 50% fat meals compared to the 25% fat meal ($p = .026$), and the 75% fat meals compared to the 25% fat meal ($p = .002$); but not between the 50% and 75% fat meals ($p = .142$). A simple main effect was found at 2 hours postprandial between 25% and 75% fat meals ($p = .027$)

and at 4 hours postprandial between 25% and 50% ($p = .017$) and 25% and 75% fat meals ($p = .013$).

Discussion

The purpose of the study was to determine if there was a dose response between HFM on plasma TG, blood biomarkers of oxidative stress, and endothelial function. In the current study, we found that the only significant change in TG occurred during the 50% fat meal at 2 hours postprandial and no significant differences with the oxidative stress measures with any of the meals or time points. We also found that on consumption of a meal consisting of higher than 25% fat, that there is a significant decrease in endothelial function within 4 hours postprandial, as measured by FMD. However, the FMD response does not appear to be related to the TG response. Our finding may have been influenced by the 1) type of subject, 2) dose of lipid 3) oxidative stress measurements and 4) measurement intervals.

Our subjects were homogeneous. We required stricter inclusion/exclusion criteria than most studies [1, 6, 12, 14-17, 20-25, 28]. All subjects were young, healthy men with no significant differences in age, height, weight, BMI, or waist circumference. There also were no significant differences in diet three months prior to testing as measured by energy intake (kcal/day), nutrient intake (total fat, saturated fat, carbohydrate, and protein) or dietary antioxidants (vitamin E and C). All subjects underwent lipid profile screening to verify that they had normal lipid values due to hyperlipidemia potentially increasing the response of HFM. We also controlled for the possible beneficial effects of physical activity that have been seen with previous research [25, 26] by including only individuals who participated in < 90 minutes of physical activity per week. Women were specifically excluded from the study based upon men responding differently to HFM than women. Premenopausal women appear to have an inherent vascular protection from HFM independent of estradiol or progesterone levels [29]. In addition, our subject size was adequate and consistent with similar research studies [1, 6, 12-17, 19, 20, 24, 25, 28-42]. Power analysis was run and determined that we would need a sample size of at least 143 people in order to show a significant difference between the 50% and 75% meals with the calculated effect size of 0.28 from our data (data not shown). Given this information, we believe that in our subject homogeneous population of young inactive healthy men, did not compromise the outcome of the TG response.

The proposed mechanism for endothelial dysfunction following a HFM is that an increase in TG leads

to increased oxidative stress which leads to decreased endothelial function. To detect a dose response for this mechanism, a dose response in TG needs to exist. However, no dose response was found for TG. The doses of lipids were chosen based the current literature. HFM with less than 40% fat did not exhibit any findings with TG, oxidative stress, and/or endothelial function [12, 40, 43, 44]. Therefore, we chose a dose of 50% to elicit a response to the HFM, the dose of 25% was chosen to be below 40% fat, and the dose of 75% was chosen to keep the same intervals in dose between meals. We also chose to control for potential confounding of calorie contents of each meal by having all meals isocaloric. Previous studies have that have investigated the effects of meals with different doses of lipids have not controlled for calories [22-24]. Therefore, it is unknown if the outcomes of these studies were based solely upon the different amounts of lipid and different caloric loads. No studies were found that have presented a dose of lipid in terms of percent total calories. It has been shown that different dietary fatty acids (stearic, palmitic, oleic, linoleic, etc.) can elicit different responses in lipid profile postprandial in young health men [45, 46]. In the current study, all fat content came from consumption of heavy whipping cream. Even with different amounts of fatty acids, the types of fatty acids would be consistent in each meal and should not affect the TG response. Perhaps the differences between doses should have been greater, such as 20%, 50%, and 80%, in order to see a dose response. Or perhaps the dose should have been in the magnitude of lipid with an increased in calories. On the other hand, when both calories and lipids are increased, the individual source (lipid or calories) for dysfunction cannot be identified. In any case, the 50% and 75% loads in our study were too close to demonstrate significant differences. Power analysis was run and determined we would need a sample size of 280 people to show significance between the 25% and 75% meals with the calculated effect size of 0.22 from our data and 2867 people for the 50% and 75% meals with the calculated effect size of 0.07 from our data (data not shown). This could also explain why there was not a dose response between oxidative stress and FMD.

The methods used to measure oxidative stress have been controversial [47, 48]. The measurement of 3-NT is appropriate for postprandial endothelial function studies due to it being an indirect marker of pro-oxidant peroxynitrite ($ONOO^-$) [49, 50]. The oxidative stress from a HFM increases the production free radicals, specifically superoxide (O_2^-), which reacts with NO to form $ONOO^-$. $ONOO^-$ is a powerful oxidant capable of oxidizing low density lipoproteins [51], of causing vascular dysfunction [52] and is responsible for nitration of tyrosine residues in proteins [49]. Therefore, the presence of nitrotyrosine (NT) in the plasma is

considered to be an indirect measure of $ONOO^-$ [49, 50]. Previous studies have successfully measured 3-NT in plasma samples [17, 50, 53, 54]. Thus, we chose to measure 3-NT due to the direct connection with vascular dysfunction.

Even though differences in oxidative stress measurement were not found, both 3-NT and TBARS have been commonly used in other postprandial oxidative stress studies [10, 16, 17, 24, 25, 31-34, 36, 50, 53, 54]. Several studies investigating postprandial oxidative stress have found changes in 3-NT within different subject population including normal controls [17, 50, 53], diabetes [17, 50, 53], and young lean healthy men [54]. 3-NT ELISA assays were used in all of these studies and were similar to the assay used in the current study. Yet, there are contractive studies [47, 48] showing that commercially available ELISA tests are not applicable for 3-NT determination in plasma samples due to technical issues and implausible results and that competitive luminescence assays are able to provide sufficient sensitivity and lead to clinically meaningful results. In the current study, we were only able to determine concentrations from a limited number of samples using 3-NT ELISA assay. It is possible that the ELISA used in our study was not a sensitive enough to determine changes in 3-NT in the plasma samples or the timing of the measurements missed the changes in oxidative stress. TBARS and/or MDA are also commonly used assay to determine postprandial oxidative stress and have been used previously in our lab [6, 12, 14, 24, 25, 32-34, 38, 39, 55-58]. In our study, there was no significant difference in TBARS found among the meals or time points.

The timing of blood samplings was based upon previous research [6, 14, 15, 17, 24, 28, 30, 37, 59] shows the peaks in TG and oxidative stress postprandial occurred at 2 and 4 hours postprandial. The timing of samplings in these studies ranged from 30 minutes to 2 hour intervals over 4-6 hours postprandial. Even though our timing of sampling should detect the peaks in TG, oxidative stress, and endothelial function, it is possible that important data between these time points were not collected. In the current study, plasma TG peaked at 2 hours postprandial but only for the 50% meal, oxidative stress did not show any significant differences, and FMD showed the largest decreased at 4 hours with the 50% and 75% meals compared to 25%. As discussed previously, the proposed mechanism is that a HFM leads to increased TG, increased oxidative stress, and decreased endothelial function. Therefore, TG and oxidative stress would increase to ultimately cause a decrease in endothelial function. Since we saw a decrease in FMD by 4 hours postprandial

in both 50% and 75% meals compared to 25%, we would assume that both TG and oxidative stress increased and could have missed the peak change due either incorrect timing of blood sampling or limited samples collected.

Even though correlations between postprandial lipemia, oxidative stress, and endothelial function have been established, there is potentially another mechanism contributing to the results. In this study, there was a decrease in FMD at 4 hours postprandial with the 50% and 75% fat meals compared with the 25% fat meal, yet no significant differences in TG or measures of oxidative stress. Since there was a change in FMD, it is possible that another mechanism could be contributing to the change which is not related to TG or the measures of oxidative stress that we measured (3-NT and MDA). Another possible explanation is that the mechanism contributed to a localized effect on the endothelium that was not detected systemically in the plasma samples. The FFA from the meal could have led to a localized increase in oxidative stress within the endothelium that decreased NO bioavailability and subsequently lead to endothelial dysfunction.

Continued research should consider the following: 1) using larger difference in doses of lipids between isocaloric meals to determine if there is a dose response with TG, oxidative stress, and endothelial function; 2) utilize different measurements of oxidative stress, such as competitive luminescence assays for 3-NT; and 3) collect more frequent measurements (such as every hour for 4 hours postprandial) to ensure the true peaks in measurements are being collected.

Conclusion

The current study found that young inactive healthy men, did not exhibit a dose response in TG for isocaloric HFMs consisting of 25%, 50%, and 75% fat. Perhaps the lipid load should be based on the amount of fat, not controlling for total calories, or larger differences in doses of lipids between the meals. Other measures of oxidative stress should be utilized before strong conclusions can be drawn. It should also be noted that even though there does not appear to be a dose response relationship of HFM, that eating meals consisting of 50% fat or higher does cause endothelial dysfunction, and should be avoided when possible.

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Table 1. Demographics of the subjects

Variable	Value
Age (yrs)	22.8 ± 2.9
Height (cm)	175.0 ± 6.0
Weight (kg)	72.80 ± 7.74
BMI (kg/m^2)	24 ± 2
Waist Circumference (cm)	80.3 ± 4.8
Physical activity (min/week)	69.5 ± 9.0

Data presented as mean ± SD

Table 2. Composition of the three challenge meals

	Meal 1	Meal 2	Meal 3
Product	16 oz Ensure® 8 oz Ensure Plus®	14 oz Ensure Plus® 2.7 oz Heavy Whipping Cream	6 oz Ensure Plus® 6 oz Heavy Whipping Cream
Calories	850 kcal	860 kcal	863 kcal
Percent Fat	25%	51%	76%
Total Fat (g)	23.0	46.3	73.3
Saturated fat (g)	3.0	20.7	42.8

Table 3. Dietary data for all subjects during the three months prior to testing

Variable	Value
Kilocalories (kcal/day)	2011.1 ± 562.2
Total fat (g)	76.9 ± 23.2
Saturated fat (g)	27.1 ± 9.1
Carbohydrate (g)	230.1 ± 77.5
Protein (g)	79.7 ± 24.5
Vitamin E (IU)	11.9 ± 3.7
Vitamin C (mg)	97.1 ± 77.2

Data presented as mean ± SD

Table 4. Baseline brachial artery diameters for the three challenge meals.

	Baseline diameters (mm)		
	25%	**50%**	**75%**
Pre-meal	3.98 ± 0.10	4.00 ± 0.31	4.08 ± 0.28
2 hours	3.99 ± 0.48	4.02 ± 0.34	3.98 ± 0.45
4 hours	3.94 ± 0.34	4.00 ± 0.43	3.98 ± 0.41

Data presented as means ± SE.

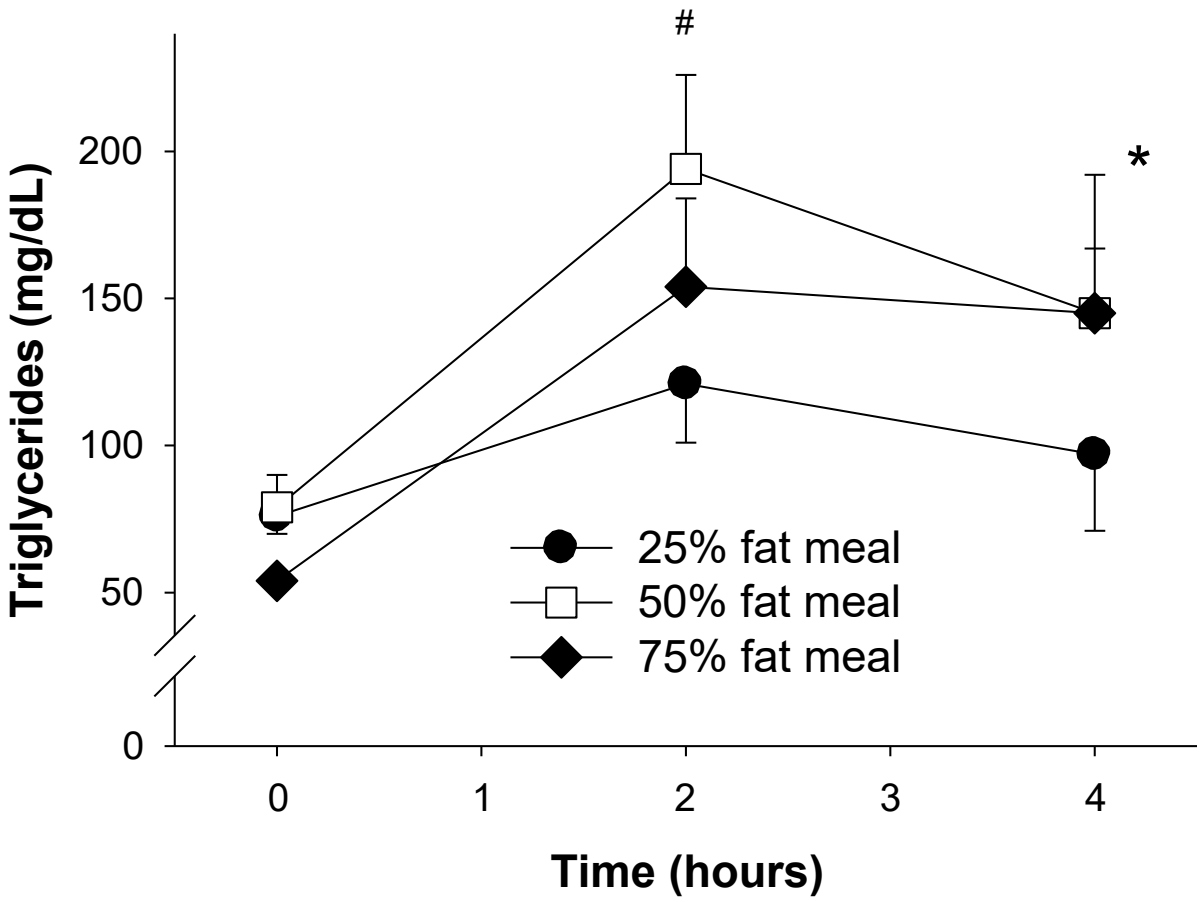

Figure 1. Blood triglycerides before and following the consumption of three challenge meals in young health men. Data presented as mean ± SEM. *indicates significant difference ($p < .05$) compared to the 25% fat meal; # indicates a significant simple main effect ($p < .05$) for the meal compared to the 25% fat meal at the time point.

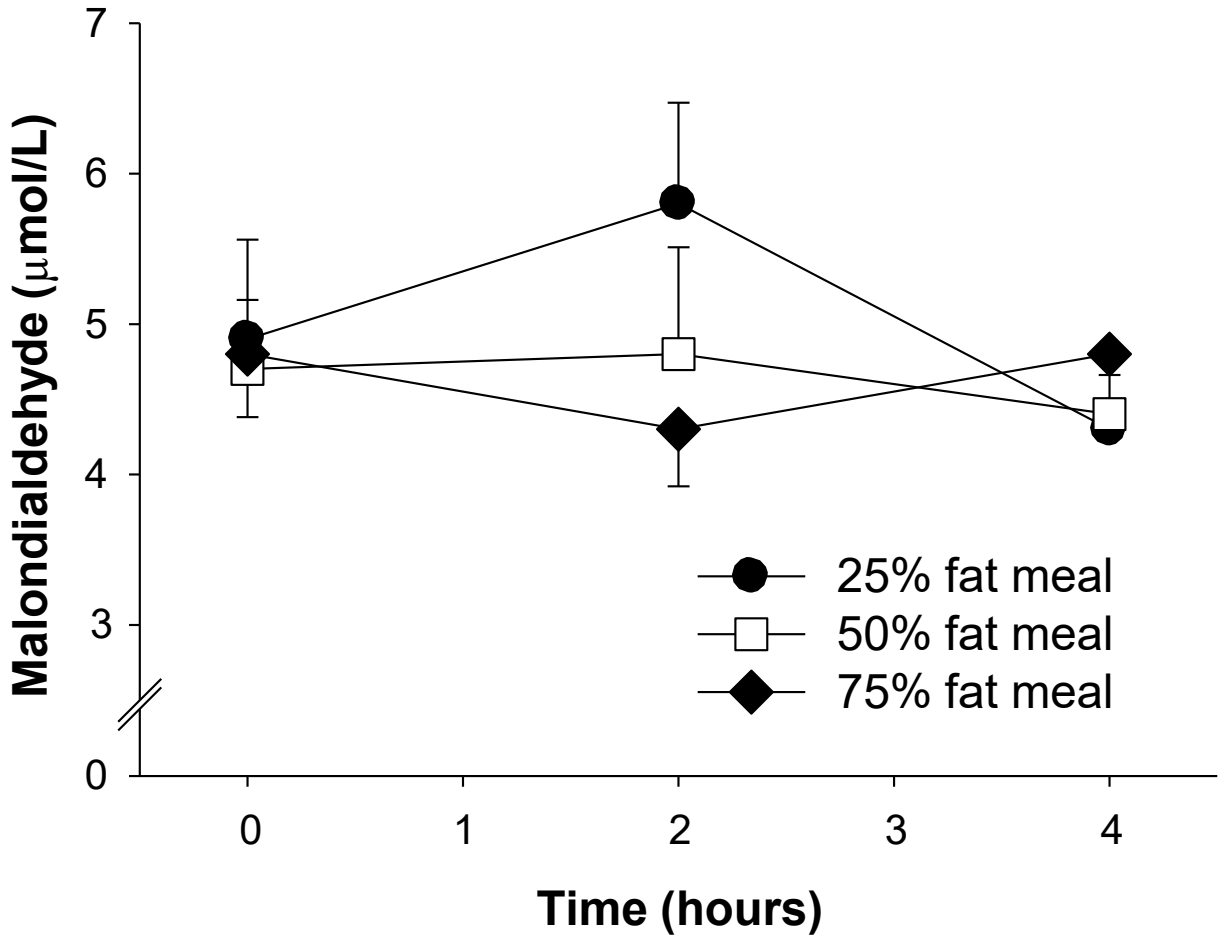

Figure 2. TBARS, as measured by malondialdehyde (MDA) concentration (uM), before and following the consumption of three challenge meals in young healthy men. Data presented as mean ± SEM.

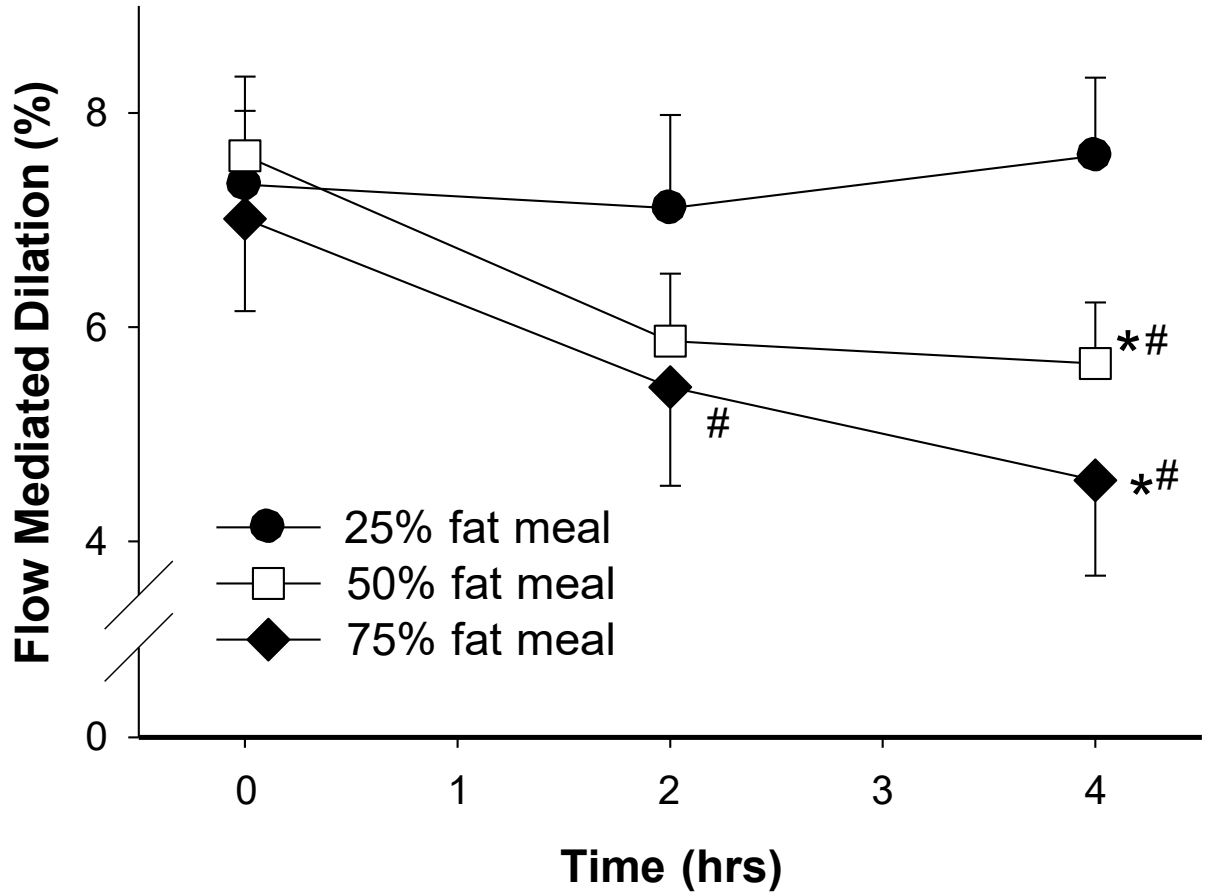

Figure 3. Flow-mediated dilation (FMD) before and following the consumption of three challenge meals in young healthy men. Data presented as mean ± SEM. *indicates significant difference ($p < .05$) compared to the 25% fat meal; # indicates significant simple main effect ($p < .05$) for the meal compared to the 25% fat meal at the time point.

Appendix A - Review of Literature

Review of Literature

I. Cardiovascular disease

Atherosclerotic cardiovascular disease (CVD) is the leading cause of morbidity and mortality in western society and will soon become the pre-eminent health problem worldwide [1, 2]. Atherosclerosis is a progressive disease that begins in childhood, advances silently through a long preclinical stage, and eventually manifest clinically, typically from middle age in western societies. This disease involves inflammatory processes and is characterized by accumulation of lipids and fibrous elements within the vasculature that eventually leads to a clinical cardiac event. Over the past several decades, it has become clear that the initiation and progression of disease, and its later activation to increase the risk of morbid events, depends on profound dynamic changes in vascular biology [8, 60]. Therefore, research should focus on what causes these changes in vascular biology and how these changes can be prevented to help decrease the risk for atherosclerosis.

There are numerous risk factors involved with atherosclerotic CVD. The risk of disease increases with age, due to the natural progression of the disease throughout life. Even though the disease affects both men and women, women have a decreased risk until menopause where their risk starts to increase due to a decreased production of estrogen [28]. Estrogen has been shown to protect the vasculature and decrease risk of CVD [31, 61, 62]. Other traditional risk factors that have been identified include smoking, physical inactivity, obesity, diabetes mellitus, high serum cholesterol concentrations, and high blood pressure. Some of the effects of obesity, physical inactivity, and diabetes mellitus are believed to be mediated by effects of insulin resistance, which influence a variety of metabolic factors associated with risk. However, a high proportion of the risk of CVD remains unexplained and recent research has focused on identifying novel risk factors to improve risk estimates and include: high C-reactive protein, a variety of measures of hemostatic function, arterial stiffness, endothelial dysfunction, inflammatory biomarkers and an exaggerated postprandial lipemic response following a high-fat meal [28]. Therefore, it is important that further research focuses on the connection between progression of CVD and these novel risk factors.

II. Endothelial function and dysfunction

The endothelium has been identified as the key regulator of vascular homeostasis, due to it not only functioning as a barrier for the vessel but also acts as an active signal transducer for circulating influences that can modify vessel wall phenotype [3]. The endothelium acts to maintain the balance between vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell proliferation and migration, and thrombogenesis and fibrinolysis [4, 5]. Certain circumstances that occur as a natural part of aging or additional perturbation (i.e. consumption of a high-fat meal), can disturb the balance. When this balance is upset, endothelial dysfunction occurs, causing damage to the arterial wall. Endothelial dysfunction is considered an early marker for atherosclerosis, preceding angiographic or ultrasound evidence of atherosclerotic plaque [5]. In order to understand the role of the endothelium in vascular disease and endothelial dysfunction, it is important to look at the normal vascular homeostasis along with progression to endothelial dysfunction.

i. Normal vascular homeostasis

The importance of the endothelium was first recognized by its effect on vascular tone. More recently, the normal, healthy endothelium has been found to regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation and vessel wall proliferation. These functions protect the endothelium from atherogenic insult. [7, 63].

The endothelium produces and releases numerous vasoconstrictors and vasodilators. There are several vasoconstrictor substances which include endothelin and angiotensin II. Endothelin has been identified as the most potent endogenous vasoconstrictor [7]. Angiotensin II not only acts as a vasoconstrictor but is also a pro-oxidant [64] and stimulates production of endothelin. Endothelin and angiotensin II promote proliferation of smooth muscle cells and there by contribute to the formation of plaque [65]. Activated macrophages and vascular smooth muscle cells, characteristic cellular components of atherosclerotic plaque, produce large amounts of endothelin.

There are also numerous vasodilator substances produced and released from the endothelium. The major vasodilator substance that is released from the endothelium is nitric oxide (NO). Other

endothelium-derived vasodilators include prostacyclin and bradykinin [65]. Prostacyclin acts synergistically with NO to inhibit platelet aggreagation [5]. Bradykinin stimulates release of NO, prostacyclin, and endothelium-derived hyperpolarizing factor, another vasodilator, which also contributes to inhibition of platelet aggreagation [65]. Bradykinin also stimulates production of tissue plasminogen activator (t-PA), and thus plays an important role in fibrinolysis.

a. Nitric Oxide (NO)

Nitric oxide (NO) is a key endothelium-derived substance essential for normal vascular homeostasis. A defect in NO production or activity has been proposed as a major mechanism of endothelial dysfunction and a contributor to atherosclerosis [7]. Therefore, it is important to look at both the production and function of NO to understand the normal role for NO and what occurs during dysfunction.

b. Production of NO

Normal NO production within the vascular endothelial cell is depicted in the figure below [12]. NO is synthesized from L-arginine, molecular oxygen (O_2), and electrons carried by NADPH and catalyzed via endothelial nitric oxide synthase (eNOS) and dependent on other cofactors (ex. tetrahydrobiopterin (BH_4), flavin adenine dinucleonide (FAD) and flavin mononucleotide (FMN)). eNOS can be activated from shear stress from arterial blood flow, insulin, and small molecule agonists (ex. acetylcholine (ACh)). Insulin and shear stress work through calcium-independent signaling pathways that are mediated in part by phosphatidylinositol-3-kinase (PI-3 kinase), while ACh works through a calcium-dependent pathway [66].

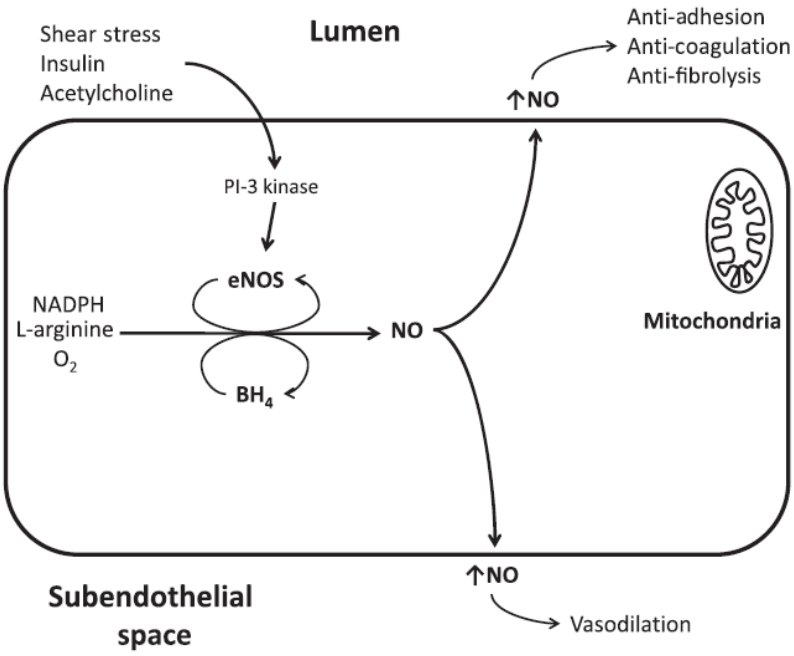

Figure 4. Normal nitric oxide production within the vascular endothelial cell. The precursors of NO include NADPH, L-arginine, and O_2 and are catalyzed by eNOS and cofactors, including BH_4. eNOS is activated by shear stress, insulin and acetylcholine increasing PI-3-kinase. Once NO is produced in the endothelium, it leaves the cell and allows for ant-adhesion, anti-coagulation, anti-fibronolysis and vasodilation. Reprinted from Wallace [12].

c. Function of NO

NO mediates endothelium-dependent vasodilation by opposing the effects of endothelium-derived vasoconstrictors such as angiotensin II and endothelin. It also inhibits platelet adherence and aggregation, leukocyte adhesion/infiltration, and proliferation of vascular smooth muscle cells. NO prevents oxidative modification of low-density lipoprotein (LDL) cholesterol [67]. Oxidation of LDL has been proposed as a major mechanism of the atherosclerotic process [68]; furthermore, plasma and macrophage content of oxidized LDL in coronary plaques correlate with severity of acute coronary syndrome [69].

Conversely, impaired production or activity of NO leads to events or action that promote atherosclerosis, such as vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion, and oxidative stress [70]. Oxidized LDL cholesterol increases synthesis of caveolin-1, which inhibits production of NO by inactivating eNOS [4]. NO normally inhibits platelet aggregation through a cyclic guanosine monophosphate (cGMP)- dependent mechanism along with inhibiting P-selectin expression on the platelet surface allowing for anti-coagulation [71, 72]. Thus, when NO bioavailability is decreased, it leads to a pro-coagulate environment and platelet aggregation. The increase in platelet aggregation also results in smooth muscle cell proliferation and migration along with vasoconstriction of the vessel.

A decrease NO bioavailability also promotes leukocyte adhesion. Leukocytes are attracted to the activated endothelium through E-selectin and chemoattractants such as monocyte chemoattractant protein-1 (MCP-1).Once the leukocytes decrease speed, the leukocyte attaches to the endothelial surface due to intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These cells can lead to atherosclerosis by evolving into macrophage and foam cells [73]. Oxidative stress can also interfere with the production and activity of NO by a number of mechanisms that are independent of LDL. For example, the free radical superoxide anion rapidly inactivates NO and destroys tetrahydrobiopterin (BH_4), a cofactor required for NO synthesis [74].

ii. Endothelial dysfunction

Endothelial dysfunction can be described as the reduction of the bioavailability of vasodilators, specifically NO, while endothelial-derived vasoconstrictors are increased [75]. This imbalance happens when damage occurs to the endothelium and initiates a number of events/processes that promote or exacerbate atherosclerosis. These include increased endothelial permeability, platelet aggregation, leukocyte adhesion, and generation of cytokines [60].Decreased production or activity of NO, manifested as impaired vasodilation, could possibly be the earliest signs of atherosclerosis. Given the relationship between endothelial dysfunction and CVD, the incidence of endothelial dysfunction may serve as a marker of unfavorable cardiovascular prognosis [76].

a. Oxidative stress

Oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium [77-79]. Oxidative stress occurs when reactive oxygen species (ROS) production is increased and/or antioxidant defenses are decreased. An important source of ROS is perhaps from the mitochondrion, where production of ROS and the dismuting capacity of mitochondrial superoxide dismutase (SOD) are carefully balanced during oxidative phosphorylation [80]. This balance can be disturbed during different situations, such as hypoxia, or conditions of increased substrate delivery, such as obesity related metabolic disorders or type II diabetes, which are characterized by hyperglycemia and hyperlipidemia [81, 82]. Other important sources of oxidative stress in the endothelium come from oxidases such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and xanthine oxidase, which have been shown on to have increased activity in arteries from patients with coronary disease [83, 84].

Oxidative stress impacts endothelial function by reducing the endothelial production and bioavailability of NO, therefore promoting cellular damage and endothelial dysfunction. [78] When mitochondria within the endothelial cell produce ROS, in the form of superoxide (O_2^-) or hydrogen peroxide (H_2O_2), it interacts to block production and decrease bioavailability of NO. eNOS uncoupling can also occur where eNOS can generate ROS and results in O_2^- formation if the cofactor BH_4 is not present, or generation of H_2O_2 if the substrate L-arginine is deficient [85]. Endothelial ROS signaling may be also be initiated by exposure to inflammatory cytokines and growth factors, and interaction of the endothelium with leukocytes [63]. Regardless of the source of oxidative stress, the interaction between ROS and NO sets up a vicious cycle, which results in further endothelial dysfunction and inflammation.

iii. Measurements of endothelial function/dysfunction

Since the discovery of importance of endothelial dysfunction in the progression of CVD, there have been numerous measurement techniques developed to be able to assess endothelial function. Ideally, the test should be safe, noninvasive, reproducible, repeatable, cost effective, and standardized

between laboratories. The results should also reflect the dynamic biology of the endothelium throughout the natural progression of atherosclerosis, define subclinical disease processes, as well as provide prognostic information for risk stratification in the later clinical phase. There is no single test that perfectly measures all these factors, yet some test are more useful at providing certain information than others [63]. Therefore, it depends on what information is trying to be obtained through testing as to which method to consider using. The table below lists the different methods of clinical assessment of endothelial function.

Table 5. Methods of clinical assessment of endothelial function. Table constructed based on information from Farouque [86].

Methods of Clinical Assessment of Endothelial Function	
Vasodilatory Function	**Coagulation Biomarkers**
Cardiac catheterization	von Willebrand Factor
Venous occlusion plethysmography	Tissue type plasminogen activator (t-PA)
Ultrasound flow-mediated dilation (FMD)	Plasminogen activator inhibitor-1 (PAI-1)
Pulse wave analysis (PWA)	Thrombomodulin
Pulse contour analysis (PCA)	**Other Biomarkers**
Pulse amplitude tonometry (PAT)	Asymmetric dimethylarginine (ADMA)
Skin Macrovascular Iontophoresis	High-sensitivity C-reactive protein (hs-CRP)
Nitric Oxide Mediators Serum	
nitrates/nitrites (NOx) **Soluble**	
Adhesion Biomarkers	
Intercellular adhesion molecule-1 (ICAM-1)	
Vascular cell adhesion molecule-1 (VCAM-1)	
E-selectin	
P-selectin	

Although there are many different methods to assess endothelial function, flow-mediated dilation (FMD) is the most frequently used method of analysis. FMD is currently the standard for noninvasive assessment of conduit artery endothelial function because there is clinical trial experience, validation, a firm link to biology, and association with cardiovascular events. FMD is measured through a noninvasive ultrasound-based test to assess conduit artery vascular function in the systemic circulation [87]. In order to understand the benefits to the technique, the procedure will be briefly outlined. The brachial artery diameter is measured before and after an increase in shear stress that is induced by reactive hyperemia. This is accomplished by placing a blood pressure cuff on the forearm distal to brachial artery and inflating the cuff to 200 mmHg or higher and releasing the cuff after 5 minutes. FMD occurs primarily as a result of local endothelial release of NO [88]. Brachial artery has been widely studied in clinical research and can be performed on almost any age group including children and the elderly. FMD also allows for testing of lifestyle and pharmacological interventions on the endothelial biology at a preclinical stage, when the disease is most likely reversible [89]. This testing can help researchers understand how the endothelium changes throughout the procession of diseases or acutely to an intervention. There are also recent guidelines that have been published to help standardize the measurements between laboratories [27].

III. Postprandial lipemia and oxidative stress

In 1979, Zilversmit proposed that atherogenesis was a postprandial phenomenon and that chylomicrons or chylomicron remnants could cause atherosclerosis [90]. Since then, many studies have produced data supporting the atherogenic effects of postprandial lipoproteins. These studies have mainly compared postprandial responses of plasma lipoproteins in patients with different forms of atherosclerosis and in control subjects without vascular disease. The data from these studies has shown that postprandial lipoprotein abnormalities have been reported in both patients with advanced clinical signs of coronary heart disease or peripheral arterial disease and with people with early markers of vascular disease such as increased intima-media thickness.

Postprandial lipemia is the result of an increase in circulating lipids in the bloodstream following a high-fat meal. Dietary fats consist mainly of triglycerides (TG) (90-98%) and are responsible for the majority of the increase in lipids in the postprandial lipemic response, with cholesterol and phospholipids

contribute only a small proportion. Therefore, the increase in plasma TG concentrations following a high-fat meal provides a good measure of postprandial lipemia. Any factor that affects the absorption, metabolism, and clearance of TG will have an effect on postprandial lipemia [28].

The average diet of North American men provides approximately 50-100 g of fat per day, consumed over 3-6 eating events [12]. Depending on the size and composition of the meal, the postprandial lipemic response can last up to 8 hours [91]. Thus, the typical North American diet results in a continuous state of postprandial lipemia.

i. Mechanism of postprandial lipemia that increase risk of CVD

Zilversmit was among the first to suggest that postprandial lipoproteins are involved in atherogenesis [90]. Numerous studies have shown that feeding animals meals high in fat produces cholesterol-rich chylomicron remnants, which are atherogenic. In a 1992 study by Patsch *et al.,* they observed that patients with coronary artery disease showed a more prolonged and pronounced postprandial TG response to a dietary fat load compared with healthy individuals [92]. Since then, numerous studies have linked the postprandial increases in plasma TG concentrations with CVD *via* effects on atherosclerosis [93, 94], either directly through the atherogenic properties of chylomicron remnants, and/or indirectly by influencing compositional changes in other lipoproteins (LDL and HDL) involved in the process of atherosclerosis. Postprandial lipemia may also increase the risk of thrombosis by increasing the activation of factor VII (FVII), which promotes blood clotting, and by decreasing fibrinolytic activity, which promotes the breakdown of blood clots [95, 96].

a. Lipoproteins

Following the hydrolysis of chylomicron TG by lipoprotein lipase (LPL), there is an accumulation of chylomicron remnant particles in the circulation. These chylomicron remnants become cholesterol-enriched as a result of transfer of cholesteryl esters in exchange for TG from HDL by cholesteryl ester transfer protein (CETP). Cholesterol-enriched remnants have pro-inflammatory and pro-atherogenic properties and are considered atherogenic [92]. There is also some evidence to suggest that chylomicron and VLDL remnants (collectively termed TG Rich Lipoprotein (TRL) remnants) are toxic to endothelial

cells due to oxidative stress [97] and may be taken up directly by subendothelial macrophages to promote foam cell formation, which are lipid-filled cells that form fatty streaks on the arterial wall. Chylomicron remnants may also promote the differentiation of white blood cells (monocytes) into macrophages [98], which enhance the process of atherosclerosis *via* the formation of foam cells. This evidence suggests that chylomicron remnants but not TG-rich chylomicrons are atherogenic. Evidence to support the role of chylomicron remnants in CVD risk comes from patients with type III hyperlipidaemia, which results in increased concentration of chylomicron remnants and is associated with increased risk of CVD. In contrast, type I hyperlipidaemia is associated with elevated chylomicron TG concentrations but there is no increase associated with an increased CVD risk [44, 49, 50].

b. Hemostatic function

Postprandial lipemia may also increase CVD risk *via* its acute effect on hemostatic function. The hemostatic system is critical in maintaining the fluid properties of the blood; it includes the coagulation pathway, platelets and the fibrinolytic system [28]. There is evidence from animal studies that postprandial lipemia increases thrombosis. In humans, Meade *et al.* were the first to show that high plasma fibrinogen, FVII coagulant activity (FVIIc) and decreased fibrinolytic activity were associated with increased risk of fatal CVD in the Northwick Park Heart Study [99]. Since then, several other hemostatic risk factors for CVD have been identified and these include: activated factor XII, plasminogen activator inhibitor-1, von Willebrand factor and decreased concentrations of soluble thrombomodulin. The BNF Task Force Report [100] also identified prothrombin fragment 1 +2, factor IX activation peptide and fibrin D-dimer (a marker of fibrin turnover) as emerging hemostatic risk factors.

Dietary lipids can affect hemostatic function in the long term and acutely after a high-fat meal [96]. Most studies investigating effects of dietary fat on hemostatic function have concentrated on their effects on the clotting factor FVII, as much of the variation in FVII levels is related to differences in fat intake. The major proportion of FVII circulates in the plasma in its inactive form (FVII). Cleavage of FVII generates the active form (FVIIa), which can now be measured using a specific and sensitive assay for FVIIa [101]. FVII coagulant activity (FVIIc) is a functional assay that measures both the inactive (FVII) and active (FVIIa) concentrations. It was found possible to produce large increases in FVIIa approximately 3-4 hours

following a meal [102]. This activation of FVII was not associated with an increase in the amount of circulating inactive FVII produced but the activation of it. Long-chain fatty acids cause an increase in FVIIa concentrations whilst short-chain and medium-chain fatty acids do not result in FVII activation [103, 104]. Different fatty acids also appear to activate FVII acutely to different degrees, with the unsaturated fatty acid oleic acid, being a particularly potent activator of FVII in the immediate 3-6 hours following a meal [105, 106]. However, there are currently few studies that have investigated the acute effects of specific fatty acids on postprandial hemostatic factors, and more studies in this area together with the mechanisms linking dietary fat and hemostatic factors recommended by the BNF Task Force Report as a priority for future research [100].

The mechanism for this activation of FVII after a fatty meal is not fully understood. While there is a relationship between fasting plasma TG concentrations and total FVII (including the activated form) [107], there appears to be no clear relationship between the extent of postprandial lipemia and FVIIa [108, 109]. Furthermore, the importance of the postprandial increase in FVIIa on CVD risk is uncertain, as in the second Northwick Park Heart Study ad relationship between FVIIc and risk of CVD was not evident and FVIIa concentration (a more sensitive marker, which was not measured in the first study) was paradoxically associated with decreased risk [110]. However, in the second Northwick Park Heart Study, assessment of FVII was made at varying times of the day in non-fasting subjects, which will have affected FVII levels. Furthermore, fat intake in the study population had fallen considerably compared with the first Northwick Park Heart Study. However, it was shown among men that there was a large increment in FVIIa following a fatty meal high in oleic acid whereas FVIIa fell following a low-fat meal. As elevated FVIIa is associated with a hypercoaguable state, it was argued that it would be prudent for older subjects who are most at risk for CVD to avoid high intakes of fat in a single meal [106].

These postprandial changes in lipids, the hemostatic system, and endothelial function enhance the progression of CVD chronically *via* effects on atherosclerosis and have acute effects *via* the process of thrombosis. Any factor influencing the magnitude or duration of postprandial lipemia will therefore also have subsequent effects on CVD risk.

ii. Factors influencing postprandial lipemia

The increase in plasma TG after consumption of a fatty meal is a function of gastric emptying, intestinal absorption, chylomicron synthesis and secretion, TG lipolysis, and chylomicron remnant removal [28]. Any factor that influences these functions will also affect the duration and magnitude of the postprandial response. Such factors include fasting TG concentrations, age, gender, apolipoprotein E genotype, weight, insulin sensitivity, LPL activity, apolipoprotein C-II, physical activity and diet. Although genetic variations (such an apolipoprotein E genotype and LPL deficiency) account for some variability observed between subjects in postprandial lipemic responses, most impaired lipemia is acquired due to factors such as diet and physical activity.

a. Physiological

Age and sex can influence the postprandial lipid response, with women exhibiting a lower response at all ages, and younger subjects a lower response compared with older subjects [111]. There is also a relationship between fasting plasma TG concentrations and the level of lipemia, which is likely to be due to competition between VLDL and chylomicrons for LPL. When the effect of fasting plasma TG is taking into account, the effects of gender and age largely disappear [111].

Both chronic and acute exercise can also affect postprandial lipemia. Exercise training [111, 112] and physical activity within the 24 hours prior to a high-fat meal [26] reduces the postprandial lipemic response. Obesity is also associated with an exaggerated postprandial lipemia, but these effects are likely to be mediated through insulin resistance, rather than overall fat mass, because fat stores are proportionately greater in women than in men. But premenopausal women show a lower postprandial lipemic response compared with men.

LPL activity is an independent predictor of postprandial TG [113] and may be the common factor in the differences in responses observed with age, sex, acute and chronic exercise and fasting TG concentrations. The activities of LPL are higher in young subjects compared with older subjects [114, 115] and there is a tendency for higher LPL activity in women compared with men [116]. Furthermore, LPL is activated by insulin and is found bound to the capillary endothelium, with the highest

concentrations in adipose tissue and muscle, where it functions to supply the underlying tissue with fatty acids derived from circulating chylomicron and VLDL.

Insulin resistance has been shown to be positively correlated to postprandial TG concentrations [117], and is believed to be an important determinant of postprandial lipemia [118]. Insulin plays a fundamental role in lipolytic activity *via* its stimulatory effects on LPL and inhibitory effects on hormone sensitive lipase (HSL) activity. Insulin resistance is associated with dyslipidaemia characterized by high VLDL concentrations, impaired postprandial clearance of chylomicrons, low HDL cholesterol concentrations and a preponderance of dense LDL particles, all associated with an increased risk of CVD. The mechanism leading to this can be explained in terms of impaired LPL activity and a failure of insulin to suppress HSL and thus free fatty acid release from adipose tissue, resulting in increased hepatic TG synthesis and VLDL synthesis and secretion. The elevated VLDL concentrations in the circulation compete with chylomicrons for lipolysis by LPL and remnant removal and thus may prolong the postprandial response [70, 71].

b. Dietary

Dietary factors, particularly habitual dietary fat composition and the amount and type of fat in a meal are major determinants of the postprandial lipemic response.

i. Test meal fatty acid composition

The magnitude of postprandial lipemia within an individual is directly proportional to the fat content of the meal [119]. Consecutive fat-containing meals also appear to enhance lipemia [114]. Short- and medium-chain SFA do not lead to significant lipemia [104, 120], because they are absorbed and transported *via* the hepatic portal vein.

Trials have shown that n-3 polyunsaturated fatty acids (PUFA) produce a reduced postprandial response compared with those from a test meal containing SFA [120-125], probably by acutely suppressing VLDL synthesis and thus reducing competition from VLDL for removal of chylomicron remnants. Studies comparing monounsaturated fatty acids (MUFA) and SFA have reported conflicting results. An early view based on small studies, utilizing small fat loads, suggested that SFA might produce

a greater postprandial response than MUFA. It has since been reported that fats rich in oleic acids (such as high-oleic sunflower oil or olive oil) have been found to cause pronounced lipemia [105, 126], with a tendency for an early peak and larger chylomicron particles. There appears to be little difference in the postprandial lipemic response between *cis*- and *trans*-MUFA [103]. Very long-chain SFAs such as arachidic, behenic, and lignoceric acids are poorly absorbed and thus have little effect on postprandial lipemia. Variable results have been obtained with fats rich in the most commonly consumed SFAs: palmitic and stearic acid. Differences in postprandial responses following fats rich in palmitric and stearic acid are believed to be due to the position of these fatty acids with the TG molecule rather than the overall fatty acid composition [30].

Differences in postprandial lipemia produced following the consumption of different fatty acids may be due to their effects on intestinal chylomicron synthesis [127], chylomicron particle size [128], the rate of chylomicron TG lipolysis [129] and chylomicron remnant removal [130], all of which have been shown to be influenced by meal fatty acid composition.

Other macronutrients within the test meal may also influence lipemia. While the protein content of a meal does not appear to influence postprandial lipemia [111], carbohydrates may affect the TG response. Indeed, it was recently reported that the presence of carbohydrate in a high-fat meal resulted in an insulin response that reduced the TG response compared with that with a low carbohydrate load [131].

ii. Background dietary fatty acid composition

Independent of any effect of the meal fatty acid composition, background dietary fatty acid composition also affects the postprandial TG response. The magnitude of postprandial lipemia is greatest following a background diet in SFA [123] while n-3 long-chain fatty acids attenuate the postprandial response to a standard fat meal [123, 124]. High intakes of long-chain n-6 fatty acids (linoleic acid) attenuate the postprandial response compared with SFA [123], but low additional intakes of linoleic acid (5 g/day) do not [132].

There appears to be no difference in postprandial lipemia following diets high in *trans*- or *cis*-MUFA [30]. A diet high in MUFA has been found to cause an early peak in plasma TG concentrations

[126]. Indeed, in 1998 Zampelas *et al.* [129] found that the pattern of lipemic response to identical meals was different in southern compared with northern Europeans and that, in particular, subjects from southern Europe (who have a higher MUFA intake compared to northern Europeans) showed a marked early rise in TG with rapid return to fasting values, whereas northern Europeans showed a slow sluggish rise in TG which did not return to fasting values until 8 or 9 hours after the meal. Furthermore, Kelly *et al.* [133] in 2001 reported that there were no adverse effects of a high-MUFA diet on fasting hemostatic factors, and found that a background diet high in MUFA resulted in a reduced postprandial activation of FVIIa in response to a standard fat-containing meal. Few studies have examined the long-term effects of diet rich in specific SFAs on postprandial lipid metabolism.

iii. How postprandial lipemia compromises protection and induces CVD.

The influx of free fatty acids (FFA) after a high-fat meal leads to advanced oxidative stress, inhibition of NO production and bioavailability which compromises the protection of the vessels. In the postprandial state, there is an exaggerated influx of FFA into the muscle, adipose, and haptic tissue [134] as well as the vascular endothelial cells [135] resulting in FFA oxidation in the mitochondria. Increased β-oxidation and oxidation of FFA-derived acetyl CoA by the tricarboxylic acid cycle creates an overproduction of electron donors (NADH and $FADH_2$), thus overloading the mitochondrial electron transport chain (ETC). As a consequence, complex III of the ETC is blocked which causes accumulation of electrons in coenzyme Q. Since coenzyme Q donates electrons to molecular oxygen this generates superoxide radicals (O_2^-) [135]. The overproduction of O_2^- results in direct and indirect effect on vascular NO bioavailability.

The resulting postprandial oxidative stress also triggers a number of atherogenic changes. These include increases in inflammation, sympathetic tone, vasoconstriction, thrombogenicity, and oxidation of low-density lipoprotein cholesterol [35, 136, 137]. The inflammatory nature of postprandial lipemia is demonstrated by immediate postprandial increases in C-reactive protein, cytokines, adhesion molecules, clotting factors, and endothelin-1. [16, 36].

IV. High-fat meals: contribution to increased risk of CVD

Research has shown that a high-fat meal is a direct source of oxidative stress [10] and postprandial lipemia may represent an independent risk factor for atherosclerotic CVD [11]. A high-fat meal leads to oxidative stress through an increase influx of free fatty acids (FFA) during the postprandial state. The FFA are broken down within the mitochondria via FFA oxidation. The increase in β-oxidation and oxidation of FFA-derived acetyl CoA by the tricarboxylic acid cycle creates an overproduction of electron donors (NADH and $FADH_2$), which overloads the mitochondrial electron transport chain (ETC). This leads to blocking complex III of the ETC causing accumulation of electrons in coenzyme Q and leads to the generation of superoxide radicals (O_2^-) [12]. The overproduction of O_2^- results in direct and indirect effects on vascular NO bioavailability, leading to an increase in endothelial dysfunction. The generation and effect of postprandial lipemia can be seen in the figures below. Based on the typical North American diet resulting in a continuous state of postprandial lipemia, the recurring postprandial oxidative stress initiates a nearly continuous cycle of endothelial dysfunction [9, 60] and leads to an increased risk factor for atherosclerotic CVD.

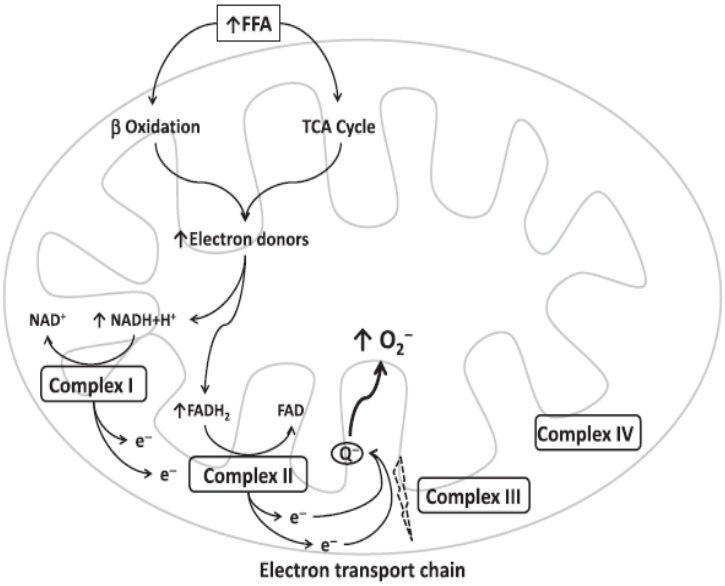

Figure 5. Mitochondrial superoxide production during postprandial lipemia. (See above text for explanation). Reprinted from Wallace [12].

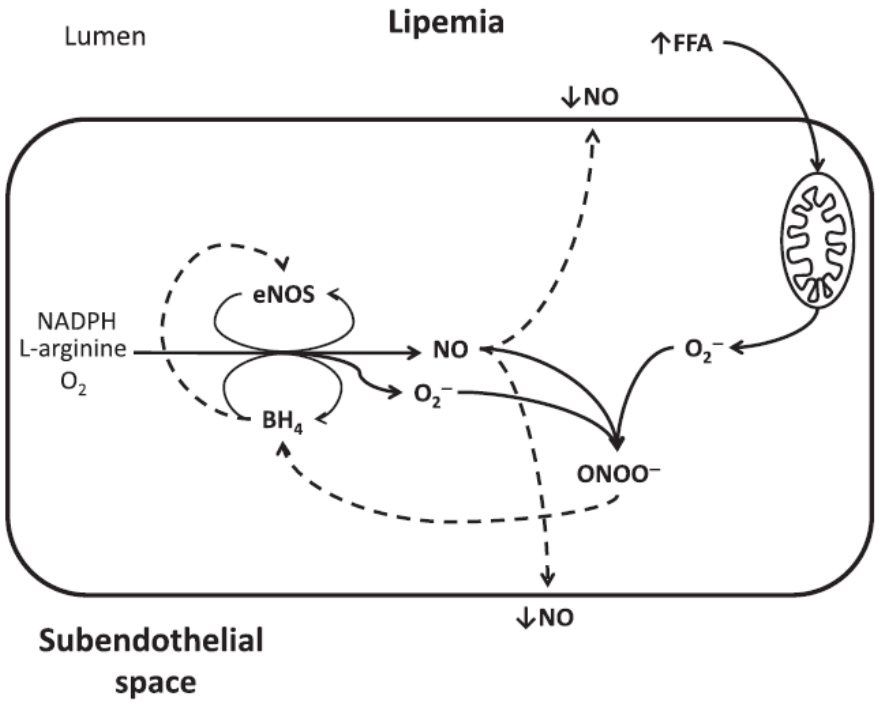

Figure 6. Nitric oxide functions in the postprandial state leading to endothelial dysfunction. During postprandial lipemia, the increase in FFA generates O_2^- from the mitochondria. O_2^- interacts with NO to not only contribute to loss of NO bioavailability from endothelial function but it also results in formation of peroxynitrite ($ONOO^-$). Furthermore, O_2 and $ONOO^-$ can oxidize BH_4, which leads to eNOS uncoupling. Uncoupled eNOS produces O_2^- instead of NO, thus resulting in a vicious cycle. Reprinted from Wallace [12]

Even with all that is known about the effect of a high-fat meal on postprandial lipemia, oxidative stress, and endothelial function, there is a gap in knowledge. There have been numerous studies that have looked at the relationship between postprandial lipemia, oxidative stress, and endothelial function during a high-fat meal [12]and even studies that have compared low-fat and high-fat meals [19-21], but there is a lack of information as to the dose response nature of high-fat meals. In recent study by Bloomer *et al.* [24], two lipids meals of 33 g (300 kcal) and 66 g (600 kcal) were given to healthy young men and TG, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) were measured pre-meal and 30 min, 1 hour, 2 hour, and 3 hour post-meal. The results showed that all values were higher after the 66 g

fat meal compared to the 33 g fat meal. The authors concluded that the magnitude of oxidative stress is dependent upon the amount of lipid consumed (66 g > 33 g). This study shows that there is an increase in oxidative stress with an increase in lipid consumption but still does not show if this occurs in a dose related fashion or the corresponding effect on endothelial function.

The significance of determining a dose response of high-fat meals is to further understand how postprandial oxidative stress relates to endothelial function and to determine if there is a ceiling effect that occurs for ROS production and subsequent oxidative stress where consumption of a certain nutrients above a certain amount may not lead to further ROS production. From this information, better recommendation could be implemented guidelines that would help people at higher risk for atherosclerotic CVD make sure their consumption of fat was at a particular level.

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Appendix B - Proposal

Postprandial lipemia, oxidative stress, and endothelial function: a dose response

Introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of morbidity and mortality in western society and will soon become the pre-eminent health problem worldwide [1, 2]. Atherosclerosis originates in the inner most cellular lining of the artery, the endothelium. The endothelium is a key regulator of vascular homeostasis, due to it not only functioning as a barrier for the vessel but through antiatherogenic functions [3]. The endothelium acts to maintain the balance between vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell proliferation and migration, and thrombogenesis and fibrinolysis [4, 5]. Certain circumstances that occur as a natural part of aging or additional perturbation (i.e. oxidative stress from consumption of a high-fat meal), can disturb the balance compromising the protective functions of the endothelium. The impairment of this vascular endothelium is called endothelial dysfunction and leads to CVD [6-9].

Research has shown that a high-fat meal is a direct source of oxidative stress [10] and postprandial lipemia may represent an independent risk factor for atherosclerotic CVD. The influx of free fatty acids (FFA) after a high-fat meal leads to advanced oxidative stress, along with inhibition of NO production and bioavailability, which compromises the protection of the vessels. Thus, postprandial oxidative stress from a high-fat meal is proposed to be the source of endothelial impairment [11]. Yet, there is a lack of research on the dose response nature of high-fat meals. The purpose of this study is to investigate the dose response relationship of three high-fat meals consisting of ~25%, ~50%, and ~75% fat on serum triglycerides (TG), blood biomarkers of oxidative stress, and endothelial function. It is hypothesized that the highest fat load will produce the greatest amount of oxidative stress and endothelial dysfunction.

Research Design

The study will be conducted as a randomized repeated measure design, along with subject blindness to the fat content of each meal. Subjects will consume three meals consisting of 25%, 51%, and 78%, over 7-10 days, with at least 1-2 days between meals. Blood samples and brachial artery flow-mediated dilation (FMD) will be performed before each meal and at 2:00 and 4:00 hours in the postprandial period.

I. Subject Selection

Ten men and ten women who participate in minimal physical activity, between the ages of 18-40 years old will be recruited for the study. Premenopausal women should have menstrual cycle duration between 25-35 days and should not be on any oral contraceptives based upon previous studies [31, 61, 62]. Women subjects should also be studied between days 1-7 of their menstrual cycle due to hormone levels being more equivalent to men during this time of the menstrual cycle. Estrogen is known to be vasoprotective [31, 61, 62].

All subjects should participate in minimal physical activity based upon previous research [25, 26]. Research has shown that healthy individuals that are insufficiently active have a greater response to a high-fat meal compared to active individuals. The Surgeon General recommends individuals participate in at least 150 minutes of physical activity weekly. Therefore, in order to categorize subjects as insufficiently active, they should participate in 90 minutes or less of physical activity weekly.

Due to the nature of the study, there are several criteria that could exclude potential subjects. These exclusion criteria include: lactose intolerance, existing coronary artery disease, existing diabetes, existing pulmonary disease, currently taking any vaso-active medications that might interfere with FMD measurements, currently taking any cholesterol lowering medication (i.e. statins), currently taking oral contraceptives, elevated cholesterol (>240 mg/dL) and/or triglycerides (>200 mg/dL),or gallbladder disease.

II. Study Procedure

Each subject will complete a screening phase before testing and three randomized high-fat meal treatments. The screening phase will include a fasting blood draw taken at the IU Health Center to exclude volunteers with high cholesterol and laboratory testing. After they qualify for the study and agree to participate, we will schedule the first meal challenge. All three meals should be consumed over a 7-10 days, with at least 1-2 days between meals. For women, this needs to be sometime within days 1-7 of their menstrual cycle [31, 61, 62].

i. Screening Phase

a. Fasting Blood Draw at IU Health Center (15-20 min)

To exclude volunteers with high cholesterol**, s**ubjects will be asked to report to the laboratory of the IU Health Center for a fasting blood draw. The sample will be 20-45 ml (4-8 ½ teaspoons) of venous blood. Blood will be drawn by a certified technician via sterile techniques for cholesterol and triglycerides. The blood draw will be analyzed for lipid profile.

The second part of the screening phase includes laboratory testing. Additional information collected as part of the screening process includes height, weight, Body Mass Index (BMI-kg/m^2) and waist circumference. Subjects will also be asked to complete a Medical History-Heath Habit Questionnaire and a (3 month) Food Frequency Questionnaire.

b. Laboratory Testing (60-90 min)

Additional information collected as part of the screening process before the three high-fat meal challenges includes height, weight, and waist circumference for each subject. Body Mass Index (kg/m^2) will be calculated using height (m) measured to the nearest 0.1 cm (stadiometer (Holtain Limited, Crymych, UK)) and weight (kg) measured to the nearest 0.05 kg (Digital Scale (Sauter, Holbrook, MA)). Both height and weight will be taken without shoes and wearing as few clothes as possible. Waist circumference will be measured using an inelastic vinyl tape measure (Creative Health Products, Ann Arbor, MI). The site for the waist will be the horizontal plane, a the level of the narrowest part of the torso, between the 10^{th} rib and the iliac crest; with the subject standing erect, with relaxed abdomen, arms by the side, and feet together. Three measurements will be taken to the nearest 0.1 cm; the average of the three measurements will be used to calculate the waist circumference.

Subjects will also be asked to complete a Medical History-Health Habit Questionnaire and a (3 month) food frequency questionnaire. The Medical History – Health Habit Questionnaire is comprised of questions on hospitalization, medications, family history and risk factors for coronary heart disease. A (3 month) food frequency questionnaire (MSEL & GSEL, Hutchinson Cancer Research Center, Nutrition Assessment Shared Resource, Seattle, WA) will be completed by subjects during screening. Variables of

interest will be energy intake (kcal/day); nutrient intake (total fat & saturated fat, carbohydrate & protein; g or mg or percent of total caloric intake), and dietary antioxidants (E & C; mg). If the subject does not qualify for the study they will have the opportunity to receive their testing results and all information will be destroyed.

ii. High-Fat Meal Challenge

Subjects will report to the Clinical Exercise Physiology lab on three separate occasions and will be expected to stay at the lab for 5-6 hours during testing. All subjects will be instructed to fast for 12 hours and abstain from caffeine, vitamin supplements (including any antioxidant), and tobacco for 12 hours before reporting to the Clinical Exercise Physiology Laboratory. In addition, each subject will be asked to abstain from physical activity/exercise 24 hours prior to the challenge meal. The procedures for the high-fat meal challenge, brachial artery FMD, and repetitive blood draws are outlined below.

a. Challenge Meals

The high-fat meal will be given between 6:00-9:00 am, depending on the subject's schedule. The three meals are summarized in Table 1 and will consist of a mixture of Ensure, Ensure Plus, and heavy whipping cream and will have a fat content of 25%, 51%, or 78%. The flavor of Ensure will be chosen by each subject. The order of the meals will be randomized for each subject, with the subject being blinded to the fat content of each meal. The meals will be prepared in the Metabolic Kitchen of the Human Performance labs prior to ingestion. The blender will be cleaned and sterilized between meals. The subject will not be allowed to eat anything except for the test meal during testing. Water will be allowed ad libidum.

	Meal 1	**Meal 2**	**Meal 3**
Product	16 oz Ensure 8 oz Ensure Plus	14 oz Ensure Plus 2.7 oz Heavy Whipping Cream	6 oz Ensure Plus 6 oz Heavy Whipping Cream
Calories	850 kcal	860 kcal	863 kcal
Percent Fat	25%	51%	78%

b. Brachial Artery FMD

Brachial artery FMD will be measured as previously described [27]. Subjects will undergo an acclimatization phase (20 min) in order to obtain hemodynamic steady state by lying supine in a dark, climate controlled room (22-24^{o}C), with their arms extended laterally. A Hokanson brachial artery cuff (Hokanson, Bellevue WA) will be placed on the subject's forearm to elicit brachial artery occlusion. The ultrasound image of the brachial artery will be obtained longitudinally 2-10 cm above the antecubital fossa by 2D high resolution Terrason t3000 (Teratech Corporation, Burlington, MA) ultrasound system, using a 7 MHz linear transducer. Baseline brachial artery diameter and Doppler flow images will be continuously recorded for 10 cardiac cycles (approx. 30 sec). Following baseline measurements, forearm occlusion will be elicited and maintained for 5 minutes by inflating the cuff to 250 mmHg. After the 5 minute occlusion, the cuff will be released and brachial artery diameter and Doppler flow images will be continuously recorded for an additional 3 minutes. The arterial diameters and blood flow velocity will be identified and measured using the Vascular Analysis Integrative System and software (Medical Imaging Applications, Coralville, Iowa). The baseline artery diameter will be compared to the maximal diameter found post occlusion, in order to determine % change in dilation. The equation for calculating percent change in FMD is as follows: ((peak hyperemic diameter – baseline diameter) / (baseline diameter)) *100.

c. Repetitive Blood Draws

Three blood draws will be collected; baseline, and 2:00 and 4:00 hours post-meal. For the purpose of collecting plasma samples, IV access will be obtained in the non-FMD arm or the back of the hand with a 22g or 24 g angiocath equipped with a PRN adapter and maintained for the duration of the treatment period. The IV access will be flushed with normal saline (Hospira Pharmaceuticals). Venous blood samples (10-20 ml) will be collected through the IV access and immediately transferred to ethylenediaminetetraacetic (EDTA) Vacutainer tubes (Vacutainer, Becton and Dickinson, Meylan, France) and separated by centrifugation within 30 minutes. Plasma will be stored in 1.0 ml aliquots at -80^{o} C until analysis. The plasma samples will be analyzed for TG, blood biomarkers of oxidative stress such as nitrotyrosine (NT), and total antioxidant capacity, using commercial assay kits.

iii. Statistical Analysis

Descriptive statistics will be used to analyze demographic data. The statistical data will be compared using two-way repeated measures ANOVA. The postprandial brachial artery FMD variable used for this analysis will be calculated as the area under the curve (AUC); using the baseline and the four hour response. Each area will be calculated as the sum of triangles and rectangles corresponding to each measurement. By using the AUC, it will give us the total volume of the responses. When ANOVA is used to test a hypothesis, Tukey HSD will be applied for follow-up to a significant F-ratio. Alpha level will be set at $p < 0.05$ for a two-tailed comparison.

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Appendix C - Raw Data

Table 6. Demographic data for all subjects who completed the study.

Subject IC	Status	Sex	008	Age	Height (em)	Height (m)	Weight (kg)	BMI	Waist (em)	PA (min/vvk)
R3162	Complete	M	6/15/1991	20.5	178.5	1.79	83.52	26	83.0	60.0
R3163	Complete	M	4/1/1990	21.8	174.0	1.74	64.78	21	74.0	85.0
R3165	Complete	M	5/9/1986	25.8	167.9	1.68	65.38	23	79.0	60.0
R3166	Complete	M	2/2/1991	21.1	162.9	1.63	66.08	25	78.0	65.0
R3167	Complete	M	9/14/1991	20.5	178.6	1.79	65.41	21	75.0	75.0
R3170	Complete	M	6/13/1983	28.8	180.1	1.80	85.52	26	88.5	75.0
R3171	Complete	M	11/26/1989	22.3	176.4	1.76	78.10	25	82.0	70.0
R3174	Complete	M	6/24/1992	19.9	179.2	1.79	74.97	23	79.0	80.0
R3177	Complete	M	8/20/1986	25.7	181.2	1.81	70.01	21	77.0	65.0
R3179	Complete	M	3/2711990	22.2	170.8	1.708	74.27	25	87.0	60.0
Average				22.8	175.0	1.75	72.80	24	80.3	69.5
St Dev				2.9	6.0	0.06	7.74	2	4.8	9.0

Table 7. Blood triglyceride concentrations (mg/dL) for all subjects before and following consumption of three challenge meals. Missing data for R3170 at 25_4 and R3179 at 75_base, 75_2, and 75_4 due to inability to draw blood at those time points.

	TG (rJ9dL)								
	25_base	25_2	25_4	50_base	50_2	50_4	75_base	75_2	75_4
R3162	77	128	67	56	209	97	50	341	219
R3163	36	77	59	60	162	99	36	108	68
R3165	261	212	112	253	292	266	130	207	347
R3166	25	28	25	20	33	22	32	57	38
R3167	41	45	40	30	115	40	41	69	60
R3170	56	105	NA	54	136	63	35	150	154
R3171	69	90	76	63	161	90	53	144	100
R3174	61	238	97	40	328	208	61	223	218
R3177	59	126	112	71	151	67	47	85	102
R3179	76	161	283	144	353	502	NA	NA	NA
Average	76	121	97	79	194	145	54	154	145
St Dev	18	63	81	36	101	148	10	95	70
St Error	6	20	26	11	32	47	3	30	22

Table 8. TBARS, as measured by malondialdehyde (MDA), concentration (umoi/L) for all subjects before and following consumption of three challenge meals. Missing data for R3170 at 25_4 and R3179 at 75_base, 75_2, and 75_4 due to inability to draw blood at those time points.

	TBARS (uM)								
	25_base	25_2	25_4	50_base	50_2	50_4	75_base	75_2	75_4
R3162	4.1	4.1	3.3	5	5.2	5.7	4.8	4.1	5.9
R3163	9.3	2.6	2.8	7.8	6.5	5.6	3.9	6.9	7
R3165	5.2	5.4	3.7	4.6	3	4.3	3.5	3.1	7.1
R3166	1.9	6	6	4.1	3.7	3.7	7.3	4.3	5.4
R3167	7.2	8.3	4.4	3.5	3.1	3.5	6.7	3.5	2.2
R3170	4.4	8.1	NA	2.9	4.8	4	4.8	5.6	3.8
R3171	5	4.4	2.7	5.8	5	3.8	4.4	4	4
R3174	4	3.7	4.2	3.7	2.5	3.7	4	3.3	3.7
R3177	4.4	6.3	5.6	3.7	4.4	4	3.7	4	3.7
R3179	3.1	8.7	6.3	5.6	10.2	5.2	NA	NA	NA
Average	4.9	5.8	4.3	4.7	4.8	4.4	4.8	4.3	4.8
SD	2.1	2.1	1.4	1.4	2.2	0.8	1.3	1.2	1.7
SE	0.66	0.67	0.43	0.46	0.71	0.26	0.42	0.38	0.53

Table 9. Flow-mediated dilation **(FMD)**, as measured by a percent change from baseline(%), for all subjects before and following consumption of three challenge meals.

	FMD (%)								
	25 base	25 2	25 4	50 base	50 2	50 4	75 base	75 2	75 4
R3162	6.13	7.91	6.9	9.59	7.97	6.21	**7.21**	**3.06**	**9.25**
R3163	5.88	4.68	6.59	5.52	4.79	4.6	**7.31**	**3.56**	**3.46**
R3165	10.89	9.8	11.78	9.25	7.7	5.37	**9.94**	**6.64**	**6.06**
R3166	8.26	6.0	4.39	5.28	2.49	5.75	**6.54**	**7.75**	**4.65**
R3167	10.18	10.95	9.97	11.13	6.81	7.79	**12.68**	**9.87**	**7.7**
R3170	6.83	5.79	5.26	6.26	5.65	4.38	**4.55**	**5.84**	**3.08**
R3171	7.77	7.36	8.02	6.61	8.46	7.67	**5.81**	**3.89**	**6.83**
R3174	3.21	3.81	5.77	5.73	4.28	2.3	**6.3**	**3.92**	**1.65**
R3177	7.01	3.85	7.64	5.69	3.69	4.64	**2.75**	**0.8**	**0.81**
R3179	7.15	10.98	9.71	10.89	6.86	7.85	**7.02**	**9.1**	**2.24**
Average	7.33	7.11	7.60	7.60	5.87	5.66	7.01	5.44	4.57
SO	2.18	2.75	2.32	2.35	2.01	1.79	2.73	2.89	2.80
SE	0.69	0.87	0.73	0.74	0.63	0.57	0.86	0.92	0.89

Table 10. Flow-mediated dilation (FMD) baseline diameters (mm) for all subjects before and following consumption of three challenge meals.

	FMD Baseline Diameter								
	25 base	25 2	25 4	50 base	50 2	50 4	75 base	75 2	75 4
R3162	3.85	3.85	3.99	4.32	3.94	3.91	4.50	4.00	3.72
R3163	4.17	4.21	4.08	3.77	3.86	4.00	4.01	4.09	4.05
R3165	3.74	3.74	3.65	3.86	3.96	3.94	3.85	3.91	4.02
R3166	3.63	3.82	3.78	3.83	3.66	3.78	3.80	3.49	3.69
R3167	3.80	3.60	3.88	3.86	3.68	3.70	3.82	3.54	3.90
R3170	4.03	4.09	4.06	4.05	4.23	4.35	4.29	4.19	4.02
R3171	3.85	3.67	3.71	3.98	4.20	3.53	3.74	3.66	3.63
R3174	4.79	5.25	4.82	4.73	4.76	5.04	4.45	4.95	5.04
R3177	3.87	3.82	3.78	3.94	4.21	4.03	4.24	4.34	4.04
R3179	4.09	3.83	3.68	3.69	3.68	3.67	4.07	3.62	3.69
Mean	3.98	3.99	3.94	4.00	4.02	4.00	4.08	3.98	3.98
SO	0.33	0.48	0.34	0.31	0.34	0.43	0.28	0.45	0.41
SE	0.10	0.15	0.11	0.10	0.11	0.14	0.09	0.14	0.13

Table 11. Flow-mediated dilation (FMD) peak diameters (mm) for all subjects before and following consumption of three challenge meals.

FMD Peak Diameter									
	25 base	25 2	25 4	50 base	50 2	50 4	75 base	75 2	75 4
R3162	4.09	4.15	4.26	4.73	4.26	4.15	4.82	4.12	4.07
R3163	4.42	4.40	4.35	3.98	4.04	4.18	4.30	4.09	4.19
R3165	4.15	4.11	4.08	4.21	4.26	4.15	4.23	4.17	4.27
R3166	3.94	4.05	3.95	4.03	3.76	4.00	4.05	3.76	3.87
R3167	4.19	3.99	4.27	4.29	3.93	3.99	4.31	3.89	4.20
R3170	4.31	4.33	4.27	4.31	4.47	4.54	4.49	4.44	4.15
R3171	4.15	3.94	4.01	4.25	4.55	3.80	3.96	3.80	3.88
R3174	4.94	5.46	5.10	5.00	4.96	5.15	4.73	5.14	5.12
R3177	4.14	3.97	4.07	4.16	4.36	4.22	4.36	4.38	4.07
R3179	4.38	4.25	4.03	4.10	3.93	3.95	4.36	3.95	3.77
Mean	4.27	4.27	4.24	4.31	4.25	4.21	4.36	4.17	4.16
SO	0.27	0.45	0.33	0.32	0.36	0.38	0.27	0.41	0.38

Appendix D - Statistics

```
GET
  FILE='E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav'.
DATASET NAME DataSet2 WINDOW=FRONT.
DATASET ACTIVATE DataSet1.
DATASET CLOSE DataSet2.
GET
  FILE='E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav'.
DATASET NAME DataSet3 WINDOW=FRONT.
DATASET ACTIVATE DataSet3.
SAVE OUTFILE='E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav'
 /COMPRESSED.
GLM TG_25_B TG_50_B TG_75_B
  /WSFACTOR=Meals 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meals) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meals.
```

General Linear Model- one-way repeated measures ANOVA for TG at baseline

[DataSet3] E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meals	Dependent Variable
1	TG_25_B
2	TG_50_B
3	TG_75_B

Descriptive Statistics

	Mean	Std. Deviation	N
TG_25_B	76.11	71.252	9
TG_50_B	71.89	69.874	9
TG_75_B	53.89	30.060	9

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meals	Pillai's Trace	.266	1.267[b]	2.000	7.000	.339
	Wilks' Lambda	.734	1.267[b]	2.000	7.000	.339
	Hotelling's Trace	.362	1.267[b]	2.000	7.000	.339
	Roy's Largest Root	.362	1.267[b]	2.000	7.000	.339

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Pillai's Trace	.266	2.534[b]	.194
	Wilks' Lambda	.266	2.534[b]	.194
	Hotelling's Trace	.266	2.534[b]	.194
	Roy's Largest Root	.266	2.534[b]	.194

a. Design: Intercept

Within Subjects Design: Meals

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meals	.304	8.334	2	.015	.590

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meals	.631	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meals

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Sphericity Assumed	2506.963	2	1253.481	1.967	.172
	Greenhouse-Geisser	2506.963	1.179	2125.847	1.967	.195
	Huynh-Feldt	2506.963	1.263	1985.175	1.967	.193
	Lower-bound	2506.963	1.000	2506.963	1.967	.198
Error(Meals)	Sphericity Assumed	10198.370	16	637.398		
	Greenhouse-Geisser	10198.370	9.434	1080.998		
	Huynh-Feldt	10198.370	10.103	1009.466		
	Lower-bound	10198.370	8.000	1274.796		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Sphericity Assumed	.197	3.933	.346
	Greenhouse-Geisser	.197	2.319	.257
	Huynh-Feldt	.197	2.483	.267
	Lower-bound	.197	1.967	.236
Error(Meals)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Linear	2222.222	1	2222.222	2.480	.154
	Quadratic	284.741	1	284.741	.752	.411
Error(Meals)	Linear	7167.778	8	895.972		
	Quadratic	3030.593	8	378.824		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Linear	.237	2.480	.284
	Quadratic	.086	.752	.120
Error(Meals)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	122277.370	1	122277.370	12.753	.007	.615
Error	76704.296	8	9588.037			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	12.753	.877
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meals

Estimates

Measure: MEASURE_1

Meals	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	76.111	23.751	21.342	130.880
2	71.889	23.291	18.179	125.599
3	53.889	10.020	30.783	76.995

Pairwise Comparisons

Measure: MEASURE_1

(I) Meals	(J) Meals	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	4.222	4.847	.409	-6.955	15.400
	3	22.222	14.110	.154	-10.317	54.761
2	1	-4.222	4.847	.409	-15.400	6.955
	3	18.000	14.224	.241	-14.802	50.802
3	1	-22.222	14.110	.154	-54.761	10.317
	2	-18.000	14.224	.241	-50.802	14.802

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.266	1.267[a]	2.000	7.000	.339	.266
Wilks' lambda	.734	1.267[a]	2.000	7.000	.339	.266
Hotelling's trace	.362	1.267[a]	2.000	7.000	.339	.266
Roy's largest root	.362	1.267[a]	2.000	7.000	.339	.266

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	2.534	.194[a]
Wilks' lambda	2.534	.194[a]
Hotelling's trace	2.534	.194[a]
Roy's largest root	2.534	.194[a]

Each F tests the multivariate effect of Meals. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM TG_25_2 TG_50_2 TG_75_2
  /WSFACTOR=Meals 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meals) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meals.
```

General Linear Model- one-way repeated measures ANOVA for TG at 2 hrs

[DataSet3] E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meals	Dependent Variable
1	TG_25_2
2	TG_50_2
3	TG_75_2

Descriptive Statistics

	Mean	Std. Deviation	N
TG_25_2	116.56	70.218	9
TG_50_2	176.33	89.766	9
TG_75_2	153.78	90.943	9

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meals	Pillai's Trace	.807	14.681[b]	2.000	7.000	.003
	Wilks' Lambda	.193	14.681[b]	2.000	7.000	.003
	Hotelling's Trace	4.194	14.681[b]	2.000	7.000	.003
	Roy's Largest Root	4.194	14.681[b]	2.000	7.000	.003

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Pillai's Trace	.807	29.361[b]	.975
	Wilks' Lambda	.807	29.361[b]	.975
	Hotelling's Trace	.807	29.361[b]	.975
	Roy's Largest Root	.807	29.361[b]	.975

a. Design: Intercept

Within Subjects Design: Meals

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meals	.440	5.739	2	.057	.641

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meals	.710	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meals

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Sphericity Assumed	16402.889	2	8201.444	4.308	.032
	Greenhouse-Geisser	16402.889	1.282	12790.313	4.308	.057
	Huynh-Feldt	16402.889	1.420	11547.590	4.308	.051
	Lower-bound	16402.889	1.000	16402.889	4.308	.072
Error(Meals)	Sphericity Assumed	30457.111	16	1903.569		
	Greenhouse-Geisser	30457.111	10.260	2968.654		
	Huynh-Feldt	30457.111	11.364	2680.216		
	Lower-bound	30457.111	8.000	3807.139		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Sphericity Assumed	.350	8.617	.663
	Greenhouse-Geisser	.350	5.525	.517
	Huynh-Feldt	.350	6.120	.549
	Lower-bound	.350	4.308	.447
Error(Meals)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Linear	6234.722	1	6234.722	2.367	.163
	Quadratic	10168.167	1	10168.167	8.670	.019
Error(Meals)	Linear	21074.778	8	2634.347		
	Quadratic	9382.333	8	1172.792		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Linear	.228	2.367	.274
	Quadratic	.520	8.670	.732
Error(Meals)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	598533.333	1	598533.333	34.296	.000	.811
Error	139616.667	8	17452.083			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	34.296	.999
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meals

Estimates

Measure: MEASURE_1

Meals	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	116.556	23.406	62.581	170.530
2	176.333	29.922	107.333	245.334
3	153.778	30.314	83.873	223.683

Pairwise Comparisons

Measure: MEASURE_1

(I) Meals	(J) Meals	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-59.778*	10.326	.000	-83.590	-35.965
	3	-37.222	24.195	.163	-93.017	18.572
2	1	59.778*	10.326	.000	35.965	83.590
	3	22.556	24.021	.375	-32.837	77.948
3	1	37.222	24.195	.163	-18.572	93.017
	2	-22.556	24.021	.375	-77.948	32.837

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.807	14.681[a]	2.000	7.000	.003	.807
Wilks' lambda	.193	14.681[a]	2.000	7.000	.003	.807
Hotelling's trace	4.194	14.681[a]	2.000	7.000	.003	.807
Roy's largest root	4.194	14.681[a]	2.000	7.000	.003	.807

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	29.361	.975[a]
Wilks' lambda	29.361	.975[a]
Hotelling's trace	29.361	.975[a]
Roy's largest root	29.361	.975[a]

Each F tests the multivariate effect of Meals. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM TG_25_4 TG_50_4 TG_75_4
  /WSFACTOR=Meals 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meals) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meals.
```

General Linear Model- one-way repeated measures ANOVA for TG at 4 hrs

[DataSet3] E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meals	Dependent Variable
1	TG_25_4
2	TG_50_4
3	TG_75_4

Descriptive Statistics

	Mean	Std. Deviation	N
TG_25_4	73.50	32.183	8
TG_50_4	111.12	83.762	8
TG_75_4	144.00	106.957	8

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meals	Pillai's Trace	.435	2.308[b]	2.000	6.000	.180
	Wilks' Lambda	.565	2.308[b]	2.000	6.000	.180
	Hotelling's Trace	.769	2.308[b]	2.000	6.000	.180
	Roy's Largest Root	.769	2.308[b]	2.000	6.000	.180

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Pillai's Trace	.435	4.617[b]	.303
	Wilks' Lambda	.435	4.617[b]	.303
	Hotelling's Trace	.435	4.617[b]	.303
	Roy's Largest Root	.435	4.617[b]	.303

a. Design: Intercept

Within Subjects Design: Meals

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meals	.536	3.742	2	.154	.683

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meals	.792	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meals

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Sphericity Assumed	19911.083	2	9955.542	4.189	.038
	Greenhouse-Geisser	19911.083	1.366	14575.422	4.189	.060
	Huynh-Feldt	19911.083	1.585	12563.879	4.189	.051
	Lower-bound	19911.083	1.000	19911.083	4.189	.080
Error(Meals)	Sphericity Assumed	33272.250	14	2376.589		
	Greenhouse-Geisser	33272.250	9.563	3479.448		
	Huynh-Feldt	33272.250	11.094	2999.252		
	Lower-bound	33272.250	7.000	4753.179		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Sphericity Assumed	.374	8.378	.637
	Greenhouse-Geisser	.374	5.722	.511
	Huynh-Feldt	.374	6.639	.558
	Lower-bound	.374	4.189	.424
Error(Meals)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			

Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Type III Sum of Squares	df	Mean Square	F	Sig.
Meals	Linear	19881.000	1	19881.000	5.104	.058
	Quadratic	30.083	1	30.083	.035	.857
Error(Meals)	Linear	27267.000	7	3895.286		
	Quadratic	6005.250	7	857.893		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meals	Partial Eta Squared	Noncent. Parameter	Observed Power
Meals	Linear	.422	5.104	.495
	Quadratic	.005	.035	.053
Error(Meals)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	287985.042	1	287985.042	19.540	.003	.736
Error	103168.625	7	14738.375			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	19.540	.964
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meals

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.435	2.308[a]	2.000	6.000	.180	.435
Wilks' lambda	.565	2.308[a]	2.000	6.000	.180	.435
Hotelling's trace	.769	2.308[a]	2.000	6.000	.180	.435
Roy's largest root	.769	2.308[a]	2.000	6.000	.180	.435

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	4.617	.303[a]
Wilks' lambda	4.617	.303[a]
Hotelling's trace	4.617	.303[a]
Roy's largest root	4.617	.303[a]

Each F tests the multivariate effect of Meals. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

Estimates

Measure: MEASURE_1

Meals	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	73.500	11.378	46.595	100.405
2	111.125	29.614	41.098	181.152
3	144.000	37.815	54.582	233.418

Pairwise Comparisons

Measure: MEASURE_1

(I) Meals	(J) Meals	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-37.625	22.929	.145	-91.844	16.594
	3	-70.500	31.206	.058	-144.291	3.291
2	1	37.625	22.929	.145	-16.594	91.844
	3	-32.875	16.819	.092	-72.645	6.895
3	1	70.500	31.206	.058	-3.291	144.291
	2	32.875	16.819	.092	-6.895	72.645

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

```
T-TEST PAIRS=TG_25 TG_25 TG_50 WITH TG_50 TG_75 TG_75 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

t-Test for TG

[DataSet3] E:\Documents\Thesis (Lipid Load)\SPSS\TG Data.sav

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TG_25	98.00	29	70.255	13.046
	TG_50	142.14	29	117.904	21.894
Pair 2	TG_25	90.38	26	63.959	12.543
	TG_75	113.73	26	89.043	17.463
Pair 3	TG_50	126.22	27	97.896	18.840
	TG_75	117.59	27	89.590	17.242

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	TG_25 & TG_50	29	.888	.000
Pair 2	TG_25 & TG_75	26	.032	.875
Pair 3	TG_50 & TG_75	27	.068	.737

Paired Samples Test

		Paired Differences				
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	
					Lower	Upper
Pair 1	TG_25 - TG_50	-44.138	64.201	11.922	-68.559	-19.717
Pair 2	TG_25 - TG_75	-23.346	107.934	21.168	-66.942	20.249
Pair 3	TG_50 - TG_75	8.630	128.139	24.660	-42.061	59.320

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	TG_25 - TG_50	-3.702	28	.001
Pair 2	TG_25 - TG_75	-1.103	25	.281
Pair 3	TG_50 - TG_75	.350	26	.729

```
GLM TBARS_25_B TBARS_50_B TBARS_75_B
  /WSFACTOR=Meal 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meal.
```

General Linear Model- one-way repeated measures ANOVA for TBARS at baseline

[DataSet2] H:\Documents\Thesis (Lipid Load)\SPSS\TBARS Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	TBARS_25_B
2	TBARS_50_B
3	TBARS_75_B

Descriptive Statistics

	Mean	Std. Deviation	N
TBARS_25_B	5.0556	2.10601	9
TBARS_50_B	4.5667	1.49164	9
TBARS_75_B	4.7889	1.34019	9

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.091	.350[b]	2.000	7.000	.716
	Wilks' Lambda	.909	.350[b]	2.000	7.000	.716
	Hotelling's Trace	.100	.350[b]	2.000	7.000	.716
	Roy's Largest Root	.100	.350[b]	2.000	7.000	.716

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.091	.701[b]	.087
	Wilks' Lambda	.091	.701[b]	.087
	Hotelling's Trace	.091	.701[b]	.087
	Roy's Largest Root	.091	.701[b]	.087

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.720	2.304	2	.316	.781

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	.936	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	1.079	2	.539	.203	.819
	Greenhouse-Geisser	1.079	1.562	.690	.203	.766
	Huynh-Feldt	1.079	1.873	.576	.203	.805
	Lower-bound	1.079	1.000	1.079	.203	.665
Error(Meal)	Sphericity Assumed	42.581	16	2.661		
	Greenhouse-Geisser	42.581	12.496	3.408		
	Huynh-Feldt	42.581	14.983	2.842		
	Lower-bound	42.581	8.000	5.323		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.025	.405	.076
	Greenhouse-Geisser	.025	.316	.073
	Huynh-Feldt	.025	.379	.075
	Lower-bound	.025	.203	.068
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	.320	1	.320	.082	.782
	Quadratic	.759	1	.759	.531	.487
Error(Meal)	Linear	31.160	8	3.895		
	Quadratic	11.421	8	1.428		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.010	.082	.057
	Quadratic	.062	.531	.099
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	623.040	1	623.040	198.819	.000	.961
Error	25.070	8	3.134			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	198.819	1.000
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	5.056	.702	3.437	6.674
2	4.567	.497	3.420	5.713
3	4.789	.447	3.759	5.819

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	.489	.568	.415	-.822	1.800
	3	.267	.930	.782	-1.879	2.412
2	1	-.489	.568	.415	-1.800	.822
	3	-.222	.765	.779	-1.987	1.542
3	1	-.267	.930	.782	-2.412	1.879
	2	.222	.765	.779	-1.542	1.987

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.091	.350[a]	2.000	7.000	.716	.091
Wilks' lambda	.909	.350[a]	2.000	7.000	.716	.091
Hotelling's trace	.100	.350[a]	2.000	7.000	.716	.091
Roy's largest root	.100	.350[a]	2.000	7.000	.716	.091

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	.701	.087[a]
Wilks' lambda	.701	.087[a]
Hotelling's trace	.701	.087[a]
Roy's largest root	.701	.087[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM TBARS_25_2 TBARS_50_2 TBARS_75_2
  /WSFACTOR=Meal 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meal.
```

General Linear Model- one-way repeated measures ANOVA for TBARS at 2 hrs

[DataSet2] H:\Documents\Thesis (Lipid Load)\SPSS\TBARS Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	TBARS_25_2
2	TBARS_50_2
3	TBARS_75_2

Descriptive Statistics

	Mean	Std. Deviation	N
TBARS_25_2	5.4333	1.94551	9
TBARS_50_2	4.2444	1.27976	9
TBARS_75_2	4.3111	1.21186	9

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.185	.795[b]	2.000	7.000	.488
	Wilks' Lambda	.815	.795[b]	2.000	7.000	.488
	Hotelling's Trace	.227	.795[b]	2.000	7.000	.488
	Roy's Largest Root	.227	.795[b]	2.000	7.000	.488

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.185	1.590[b]	.138
	Wilks' Lambda	.185	1.590[b]	.138
	Hotelling's Trace	.185	1.590[b]	.138
	Roy's Largest Root	.185	1.590[b]	.138

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.201	11.218	2	.004	.556

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	.581	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	8.032	2	4.016	1.712	.212
	Greenhouse-Geisser	8.032	1.112	7.223	1.712	.226
	Huynh-Feldt	8.032	1.163	6.909	1.712	.226
	Lower-bound	8.032	1.000	8.032	1.712	.227
Error(Meal)	Sphericity Assumed	37.541	16	2.346		
	Greenhouse-Geisser	37.541	8.896	4.220		
	Huynh-Feldt	37.541	9.300	4.037		
	Lower-bound	37.541	8.000	4.693		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.176	3.423	.306
	Greenhouse-Geisser	.176	1.903	.223
	Huynh-Feldt	.176	1.990	.228
	Lower-bound	.176	1.712	.211
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			

Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	5.667	1	5.667	1.805	.216
	Quadratic	2.365	1	2.365	1.523	.252
Error(Meal)	Linear	25.118	8	3.140		
	Quadratic	12.424	8	1.553		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.184	1.805	.220
	Quadratic	.160	1.523	.193
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	587.067	1	587.067	267.006	.000	.971
Error	17.590	8	2.199			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	267.006	1.000
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	5.433	.649	3.938	6.929
2	4.244	.427	3.261	5.228
3	4.311	.404	3.380	5.243

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	1.189	.898	.222	-.882	3.260
	3	1.122	.835	.216	-.804	3.048
2	1	-1.189	.898	.222	-3.260	.882
	3	-.067	.244	.792	-.630	.497
3	1	-1.122	.835	.216	-3.048	.804
	2	.067	.244	.792	-.497	.630

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.185	.795[a]	2.000	7.000	.488	.185
Wilks' lambda	.815	.795[a]	2.000	7.000	.488	.185
Hotelling's trace	.227	.795[a]	2.000	7.000	.488	.185
Roy's largest root	.227	.795[a]	2.000	7.000	.488	.185

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	1.590	.138[a]
Wilks' lambda	1.590	.138[a]
Hotelling's trace	1.590	.138[a]
Roy's largest root	1.590	.138[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM TBARS_25_4 TBARS_50_4 TBARS_75_4
  /WSFACTOR=Meal 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
```

/CRITERIA=ALPHA(.05)

/WSDESIGN=Meal.

General Linear Model- one-way repeated measures ANOVA for TBARS at 4 hrs

[DataSet2] H:\Documents\Thesis (Lipid Load)\SPSS\TBARS Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	TBARS_25_4
2	TBARS_50_4
3	TBARS_75_4

Descriptive Statistics

	Mean	Std. Deviation	N
TBARS_25_4	4.0875	1.21941	8
TBARS_50_4	4.2875	.87413	8
TBARS_75_4	4.8750	1.75153	8

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.190	.705[b]	2.000	6.000	.531
	Wilks' Lambda	.810	.705[b]	2.000	6.000	.531
	Hotelling's Trace	.235	.705[b]	2.000	6.000	.531

	Roy's Largest Root	.235	.705[b]	2.000	6.000	.531

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.190	1.410[b]	.122
	Wilks' Lambda	.190	1.410[b]	.122
	Hotelling's Trace	.190	1.410[b]	.122
	Roy's Largest Root	.190	1.410[b]	.122

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.543	3.661	2	.160	.686

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	.798	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	2.681	2	1.340	.728	.500
	Greenhouse-Geisser	2.681	1.373	1.953	.728	.458
	Huynh-Feldt	2.681	1.596	1.679	.728	.475
	Lower-bound	2.681	1.000	2.681	.728	.422
Error(Meal)	Sphericity Assumed	25.766	14	1.840		
	Greenhouse-Geisser	25.766	9.610	2.681		
	Huynh-Feldt	25.766	11.175	2.306		

Lower-bound	25.766	7.000	3.681		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.094	1.457	.149
	Greenhouse-Geisser	.094	1.000	.129
	Huynh-Feldt	.094	1.163	.136
	Lower-bound	.094	.728	.115
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	2.481	1	2.481	.832	.392
	Quadratic	.200	1	.200	.287	.609
Error(Meal)	Linear	20.874	7	2.982		
	Quadratic	4.891	7	.699		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.106	.832	.124
	Quadratic	.039	.287	.075
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	468.167	1	468.167	285.799	.000	.976
Error	11.467	7	1.638			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	285.799	1.000

Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	4.088	.431	3.068	5.107
2	4.288	.309	3.557	5.018
3	4.875	.619	3.411	6.339

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-.200	.651	.768	-1.740	1.340
	3	-.787	.863	.392	-2.829	1.254
2	1	.200	.651	.768	-1.340	1.740
	3	-.588	.459	.241	-1.672	.497
3	1	.787	.863	.392	-1.254	2.829
	2	.588	.459	.241	-.497	1.672

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.190	.705[a]	2.000	6.000	.531	.190
Wilks' lambda	.810	.705[a]	2.000	6.000	.531	.190
Hotelling's trace	.235	.705[a]	2.000	6.000	.531	.190
Roy's largest root	.235	.705[a]	2.000	6.000	.531	.190

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	1.410	.122[a]
Wilks' lambda	1.410	.122[a]
Hotelling's trace	1.410	.122[a]
Roy's largest root	1.410	.122[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

DATASET ACTIVATE DataSet2.

DATASET CLOSE DataSet3.

T-TEST PAIRS=TBARS_25 TBARS_25 TBARS_50 WITH TBARS_50 TBARS_75 TBARS_75 (PAIRED)

/CRITERIA=CI(.9500)

/MISSING=ANALYSIS.

t-Test for TBARS

[DataSet2] H:\Documents\Thesis (Lipid Load)\SPSS\TBARS Data.sav

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TBARS_25	5.0069	29	1.92297	.35709
	TBARS_50	4.6414	29	1.59071	.29539
Pair 2	TBARS_25	4.9654	26	2.00817	.39383
	TBARS_75	4.6538	26	1.40035	.27463
Pair 3	TBARS_50	4.6556	27	1.63668	.31498
	TBARS_75	4.6185	27	1.38537	.26662

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	TBARS_25 & TBARS_50	29	.282	.139
Pair 2	TBARS_25 & TBARS_75	26	.106	.605
Pair 3	TBARS_50 & TBARS_75	27	.231	.246

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower
Pair 1	TBARS_25 - TBARS_50	.36552	2.12254	.39415	-.44186
Pair 2	TBARS_25 - TBARS_75	.31154	2.32281	.45554	-.62667
Pair 3	TBARS_50 - TBARS_75	.03704	1.88397	.36257	-.70824

Paired Samples Test

		Paired Differences	t	df	Sig. (2-tailed)
		95% Confidence Interval of the Difference Upper			
Pair 1	TBARS_25 - TBARS_50	1.17289	.927	28	.362
Pair 2	TBARS_25 - TBARS_75	1.24974	.684	25	.500
Pair 3	TBARS_50 - TBARS_75	.78231	.102	26	.919

```
GET
  FILE='E:\Documents\Thesis (Lipid Load)\SPSS\FMD Data.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
GLM FMD_25_B FMD_50_B FMD_75_B
  /WSFACTOR=Meal 3 Polynomial
  /METHOD=SSTYPE(3)
```

/EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)

/PRINT=DESCRIPTIVE ETASQ OPOWER

/CRITERIA=ALPHA(.05)

/WSDESIGN=Meal.

General Linear Model- one-way repeated measures ANOVA for FMD at baseline

[DataSet1] E:\Documents\Thesis (Lipid Load)\SPSS\FMD Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	FMD_25_B
2	FMD_50_B
3	FMD_75_B

Descriptive Statistics

	Mean	Std. Deviation	N
FMD_25_B	7.3310	2.17961	10
FMD_50_B	7.5950	2.34750	10
FMD_75_B	7.0110	2.73309	10

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.084	.365[b]	2.000	8.000	.705
	Wilks' Lambda	.916	.365[b]	2.000	8.000	.705
	Hotelling's Trace	.091	.365[b]	2.000	8.000	.705
	Roy's Largest Root	.091	.365[b]	2.000	8.000	.705

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.084	.729[b]	.090
	Wilks' Lambda	.084	.729[b]	.090
	Hotelling's Trace	.084	.729[b]	.090
	Roy's Largest Root	.084	.729[b]	.090

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.973	.217	2	.897	.974

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	1.711	2	.855	.344	.713
	Greenhouse-Geisser	1.711	1.948	.878	.344	.708
	Huynh-Feldt	1.711	2.000	.855	.344	.713
	Lower-bound	1.711	1.000	1.711	.344	.572
Error(Meal)	Sphericity Assumed	44.704	18	2.484		
	Greenhouse-Geisser	44.704	17.530	2.550		
	Huynh-Feldt	44.704	18.000	2.484		
	Lower-bound	44.704	9.000	4.967		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.037	.689	.097
	Greenhouse-Geisser	.037	.671	.096
	Huynh-Feldt	.037	.689	.097
	Lower-bound	.037	.344	.082
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			

	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	.512	1	.512	.188	.675
	Quadratic	1.199	1	1.199	.535	.483
Error(Meal)	Linear	24.525	9	2.725		
	Quadratic	20.179	9	2.242		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.020	.188	.068
	Quadratic	.056	.535	.101
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1604.107	1	1604.107	125.673	.000	.933
Error	114.877	9	12.764			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	125.673	1.000
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	7.331	.689	5.772	8.890
2	7.595	.742	5.916	9.274
3	7.011	.864	5.056	8.966

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-.264	.727	.725	-1.910	1.382
	3	.320	.738	.675	-1.350	1.990
2	1	.264	.727	.725	-1.382	1.910
	3	.584	.645	.389	-.875	2.043
3	1	-.320	.738	.675	-1.990	1.350
	2	-.584	.645	.389	-2.043	.875

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.084	.365[a]	2.000	8.000	.705	.084
Wilks' lambda	.916	.365[a]	2.000	8.000	.705	.084
Hotelling's trace	.091	.365[a]	2.000	8.000	.705	.084
Roy's largest root	.091	.365[a]	2.000	8.000	.705	.084

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	.729	.090[a]
Wilks' lambda	.729	.090[a]
Hotelling's trace	.729	.090[a]
Roy's largest root	.729	.090[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM FMD_25_2 FMD_50_2 FMD_75_2
 /WSFACTOR=Meal 3 Polynomial
 /METHOD=SSTYPE(3)
 /EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)
```

/PRINT=DESCRIPTIVE ETASQ OPOWER

/CRITERIA=ALPHA(.05)

/WSDESIGN=Meal.

General Linear Model- one-way repeated measures ANOVA for FMD at 2 hrs

[DataSet1] E:\Documents\Thesis (Lipid Load)\SPSS\FMD Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	FMD_25_2
2	FMD_50_2
3	FMD_75_2

Descriptive Statistics

	Mean	Std. Deviation	N
FMD_25_2	7.1130	2.74991	10
FMD_50_2	5.8700	2.00694	10
FMD_75_2	5.4430	2.89440	10

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.635	6.968[b]	2.000	8.000	.018
	Wilks' Lambda	.365	6.968[b]	2.000	8.000	.018
	Hotelling's Trace	1.742	6.968[b]	2.000	8.000	.018

	Roy's Largest Root	1.742	6.968[b]	2.000	8.000	.018

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.635	13.936[b]	.788
	Wilks' Lambda	.635	13.936[b]	.788
	Hotelling's Trace	.635	13.936[b]	.788
	Roy's Largest Root	.635	13.936[b]	.788

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.498	5.571	2	.062	.666

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	.738	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	15.054	2	7.527	2.402	.119
	Greenhouse-Geisser	15.054	1.332	11.303	2.402	.143
	Huynh-Feldt	15.054	1.476	10.199	2.402	.138
	Lower-bound	15.054	1.000	15.054	2.402	.156
Error(Meal)	Sphericity Assumed	56.418	18	3.134		
	Greenhouse-Geisser	56.418	11.987	4.707		
	Huynh-Feldt	56.418	13.285	4.247		

Lower-bound	56.418	9.000	6.269		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.211	4.803	.421
	Greenhouse-Geisser	.211	3.199	.332
	Huynh-Feldt	.211	3.545	.352
	Lower-bound	.211	2.402	.284
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	13.945	1	13.945	6.973	.027
	Quadratic	1.110	1	1.110	.260	.622
Error(Meal)	Linear	17.997	9	2.000		
	Quadratic	38.420	9	4.269		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.437	6.973	.653
	Quadratic	.028	.260	.074
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1131.725	1	1131.725	82.615	.000	.902
Error	123.289	9	13.699			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	82.615	1.000

Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	7.113	.870	5.146	9.080
2	5.870	.635	4.434	7.306
3	5.443	.915	3.372	7.514

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	1.243	.640	.084	-.205	2.691
	3	1.670*	.632	.027	.239	3.101
2	1	-1.243	.640	.084	-2.691	.205
	3	.427	1.035	.690	-1.914	2.768
3	1	-1.670*	.632	.027	-3.101	-.239
	2	-.427	1.035	.690	-2.768	1.914

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.635	6.968[a]	2.000	8.000	.018	.635
Wilks' lambda	.365	6.968[a]	2.000	8.000	.018	.635
Hotelling's trace	1.742	6.968[a]	2.000	8.000	.018	.635
Roy's largest root	1.742	6.968[a]	2.000	8.000	.018	.635

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	13.936	.788[a]
Wilks' lambda	13.936	.788[a]
Hotelling's trace	13.936	.788[a]
Roy's largest root	13.936	.788[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
GLM FMD_25_4 FMD_50_4 FMD_75_4
  /WSFACTOR=Meal 3 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Meal) COMPARE ADJ(LSD)
  /PRINT=DESCRIPTIVE ETASQ OPOWER
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Meal.
```

General Linear Model- one-way repeated measures ANOVA for FMD at 4 hrs

[DataSet1] E:\Documents\Thesis (Lipid Load)\SPSS\FMD Data.sav

Within-Subjects Factors

Measure: MEASURE_1

Meal	Dependent Variable
1	FMD_25_4
2	FMD_50_4
3	FMD_75_4

Descriptive Statistics

	Mean	Std. Deviation	N
FMD_25_4	7.6030	2.31800	10
FMD_50_4	5.6560	1.79071	10
FMD_75_4	4.5730	2.80037	10

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Meal	Pillai's Trace	.549	4.866[b]	2.000	8.000	.041
	Wilks' Lambda	.451	4.866[b]	2.000	8.000	.041
	Hotelling's Trace	1.217	4.866[b]	2.000	8.000	.041
	Roy's Largest Root	1.217	4.866[b]	2.000	8.000	.041

Multivariate Tests[a]

Effect		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Pillai's Trace	.549	9.733[b]	.628
	Wilks' Lambda	.549	9.733[b]	.628
	Hotelling's Trace	.549	9.733[b]	.628
	Roy's Largest Root	.549	9.733[b]	.628

a. Design: Intercept

Within Subjects Design: Meal

b. Exact statistic

c. Computed using alpha = .05

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser
Meal	.758	2.220	2	.330	.805

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Epsilon	
	Huynh-Feldt	Lower-bound
Meal	.954	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.[a]

a. Design: Intercept

Within Subjects Design: Meal

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Sphericity Assumed	47.149	2	23.574	7.228	.005
	Greenhouse-Geisser	47.149	1.610	29.287	7.228	.009
	Huynh-Feldt	47.149	1.908	24.713	7.228	.006
	Lower-bound	47.149	1.000	47.149	7.228	.025
Error(Meal)	Sphericity Assumed	58.708	18	3.262		
	Greenhouse-Geisser	58.708	14.489	4.052		
	Huynh-Feldt	58.708	17.170	3.419		
	Lower-bound	58.708	9.000	6.523		

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Sphericity Assumed	.445	14.456	.888
	Greenhouse-Geisser	.445	11.636	.826
	Huynh-Feldt	.445	13.790	.875
	Lower-bound	.445	7.228	.668
Error(Meal)	Sphericity Assumed			
	Greenhouse-Geisser			

	Huynh-Feldt			
	Lower-bound			

a. Computed using alpha = .05

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Type III Sum of Squares	df	Mean Square	F	Sig.
Meal	Linear	45.904	1	45.904	9.493	.013
	Quadratic	1.244	1	1.244	.737	.413
Error(Meal)	Linear	43.521	9	4.836		
	Quadratic	15.187	9	1.687		

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	Meal	Partial Eta Squared	Noncent. Parameter	Observed Power
Meal	Linear	.513	9.493	.783
	Quadratic	.076	.737	.120
Error(Meal)	Linear			
	Quadratic			

a. Computed using alpha = .05

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1059.934	1	1059.934	107.078	.000	.922
Error	89.088	9	9.899			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Noncent. Parameter	Observed Power
Intercept	107.078	1.000
Error		

a. Computed using alpha = .05

Estimated Marginal Means

Meal

Estimates

Measure: MEASURE_1

Meal	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	7.603	.733	5.945	9.261
2	5.656	.566	4.375	6.937
3	4.573	.886	2.570	6.576

Pairwise Comparisons

Measure: MEASURE_1

(I) Meal	(J) Meal	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	1.947*	.663	.017	.446	3.448
	3	3.030*	.983	.013	.805	5.255
2	1	-1.947*	.663	.017	-3.448	-.446
	3	1.083	.741	.178	-.594	2.760
3	1	-3.030*	.983	.013	-5.255	-.805
	2	-1.083	.741	.178	-2.760	.594

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.549	4.866[a]	2.000	8.000	.041	.549
Wilks' lambda	.451	4.866[a]	2.000	8.000	.041	.549
Hotelling's trace	1.217	4.866[a]	2.000	8.000	.041	.549
Roy's largest root	1.217	4.866[a]	2.000	8.000	.041	.549

Multivariate Tests

	Noncent. Parameter	Observed Power
Pillai's trace	9.733	.628[a]
Wilks' lambda	9.733	.628[a]
Hotelling's trace	9.733	.628[a]
Roy's largest root	9.733	.628[a]

Each F tests the multivariate effect of Meal. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

b. Computed using alpha = .05

```
T-TEST PAIRS=FMD_25 FMD_25 FMD_50 WITH FMD_50 FMD_75 FMD_75 (PAIRED)
 /CRITERIA=CI(.9500)
 /MISSING=ANALYSIS.
```

t-Test for FMD

[DataSet1] E:\Documents\Thesis (Lipid Load)\SPSS\FMD Data.sav

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	FMD_25	7.3490	30	2.35165	.42935
	FMD_50	6.3737	30	2.17597	.39728
Pair 2	FMD_25	7.3490	30	2.35165	.42935
	FMD_75	5.6757	30	2.89908	.52930
Pair 3	FMD_50	6.3737	30	2.17597	.39728
	FMD_75	5.6757	30	2.89908	.52930

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	FMD_25 & FMD_50	30	.499	.005
Pair 2	FMD_25 & FMD_75	30	.494	.006
Pair 3	FMD_50 & FMD_75	30	.534	.002

Paired Samples Test

		Paired Differences				
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	
					Lower	Upper
Pair 1	FMD_25 - FMD_50	.97533	2.27161	.41474	.12710	1.82357
Pair 2	FMD_25 - FMD_75	1.67333	2.68333	.48991	.67136	2.67531

Pair 3	FMD_50 - FMD_75	.69800	2.52995	.46190	-.24670	1.64270

Paired Samples Test

		t	df	Sig. (2-tailed)
Pair 1	FMD_25 - FMD_50	2.352	29	.026
Pair 2	FMD_25 - FMD_75	3.416	29	.002
Pair 3	FMD_50 - FMD_75	1.511	29	.142

Appendix E – Informed Consent

IRB Study #0907000527

INDIANA UNIVERSITY BLOOMINGTON

INFORMED CONSENT STATEMENT

Lipemia, oxidative stress and endothelial function: a dose response.

You are invited to participate in a research study of how much high-fat meals affect your artery health. You were selected as a possible subject because you are a healthy adult between 18 and 40 years of age. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

The study is being conducted by Janet P. Wallace, Ph.D., and Sylvanna Bielko, B.A., from the Clinical Exercise Physiology Laboratory, Department of Kinesiology, School of Health, Physical Education and Recreation, Indiana University, Bloomington. This study is not currently funded.

STUDY PURPOSE

The purpose of this study is to find the relationship between the fat content of a meal and its effects on artery health and oxidative stress.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you agree to participate, you will be one of twenty subjects who will be participating in this research.

PROCEDURES FOR THE STUDY:

If you agree to be in the study, you will be asked to participate in screening, laboratory testing, and three high-fat meals. Each meal will have a different fat content; ranging from 25% to 75%. The following procedures will be done at each meal challenge. There will be three separate meal challenges over a 7-10 day period:

1. Screening

a. Blood Draw at IU Health Center (20-30 min)

Screening blood draw for cholesterol and triglycerides will be taken at the IU Health Center.

Approximately 20-45 ml (4-8 ½ teaspoons) of venous blood will be drawn by a certified technician via sterile techniques.

2. Laboratory Testing <u>(60-90 min)</u>

The laboratory testing will occur on a separate day before scheduling the three high-fat meals.

a. Medical History – Health Habit Questionnaire
You will be asked to complete a questionnaire on hospitalization, medications, family history and risk factors for coronary heart disease.

b. Basic Laboratory Testing

c. Body Mass Index (kg/m^2) will be calculated using height and weight. Both height and weight will be taken without shoes and wearing as few clothes as possible.

d. Waist circumference will be measured using an inelastic vinyl tape measure. The site for the waist will be the horizontal plane, a the level of the narrowest part of the torso, between the 10^{th} rib and the iliac crest; while you are standing erect, with relaxed abdomen, arms by the side, and feet together. Three measurements will be taken to the nearest 0.1 cm; the average of the three measurements will be used to calculate the waist circumference.

e. Nutrition/Diet: A 3 month food frequency questionnaire will be analyzed for caloric intake (kcal/day); total fat & saturated fat, carbohydrate & protein; (g or mg or percent of total caloric intake), and dietary antioxidants (E & C; mg).

If you do not qualify for the study based on your results from either the screening blood draw or the laboratory testing, you will have the opportunity to receive the results. If you do not want the results, they will be destroyed. You will not receive payment for completing the screening blood draw or laboratory testing; payment is only for completing the high-fat meals.

3. High-Fat Meal Challenges

a. **High- Fat Meal** - You will eat a high-fat breakfast meal consisting of mixtures of Ensure and whipping cream or heavy whipping cream. The flavor of Ensure will be your choice (, Homemade Vanilla, Creamy Milk Chocolate, Strawberries & Cream, or Butter Pecan):

The meals will be given in a randomized order, meaning you will not necessarily receive in 1-2-3 order. Each meal will be provided on a separate day, which could take up to 5 hours to complete.

	Meal 1	**Meal 2**	**Meal 3**
Product	16 oz Ensure 8 oz Ensure Plus	14 oz Ensure Plus 2.7 oz Heavy Whipping Cream	6 oz Ensure Plus 6 oz Heavy Whipping Cream
Calories	850 kcal	860 kcal	863 kcal
Percent Fat	25%	51%	78%

Water will be allowed as needed. Consuming the meal may take between 5-20 minutes.

b. **Pre- & Post-Meal Blood Draws** - Three blood draws (to determine the amount of fat in the meal) will be measured 1) at baseline and 2) at 2:00 and 4:00 hours following the meal.

A 22-24 gauage venous (vein) catheter will be inserted and remain in your left arm by a trained clinical exercise physiologist for approximately seven hours. Following each blood draw, the catheter will be flushed (cleaned) with normal saline. The total amount of blood drawn for the three blood draws will be 105-140 ml (21-28 teaspoons). Inserting the catheter should take 5-10 minutes and each blood draw should take 1-2 minutes.

c. **Brachial Artery Flow-Mediated Dilation** (FMD) - *For the Measurement of Arterial Health*

To participate in the high-fat meals you must:

- Not exercise for at least 12 hrs before each study
- Not have any caffeine at least 8 hrs before each study
- Not have any Vitamin supplementation for at least 8 hrs before each study
- Not have any tobacco products at least 8 hrs before each study
- Be fasting for the past 12 hrs
- If you are on any medications that dilate your arteries and/or cannot be tested within the preparation criteria listed above you will be excluded from the study

How well your artery expands with changes in blood flow is used to characterize the health of your arteries. We will perform the FMD measurement on your right arm, before and at 2 hours and 4 hours after the high-fat meal. Three EKG-electrodes will be placed on your chest and you will lie on your back in a dark, climate-controlled room (22-24^{o}C or 72-75^{o}F) with both arms extended out to your sides. After resting for 20 minutes, an ultrasound scan of your upper arm will be taken. This may require several scans to obtain a clear image. Once a clear image has been obtained, a blood pressure cuff will be placed around your forearm and inflated to 250 mmHg to stop blood flow to your lower arm for 5 minutes. The cuff will then be deflated and we will measure your blood flow velocity (the speed at which the blood is flowing through the artery) for 10 seconds and additional ultrasounds scans will be taken for additional 2 minutes. The health of your artery will be expressed as a percentages of artery expansion with the increased blood flow, compared to the resting diameter.

RISKS OF TAKING PART IN THE STUDY:

While participating in this study, the risks and discomforts are minimal:

1. The risk associated with a **fasting blood draw** may include fainting, soreness, bruising, and/or swelling at the venipuncture site (area where venous (vein) catheter was located).
2. **Challenge Meal:** The fat content of this meal is consistent with the fat content of the American diet. Diarrhea and/or stomach cramps may occur if you are not used to eating a high fat diet (50% high-fat meals). The high-fat meal will be prepared in the Metabolic Kitchen of the Human Performance Laboratory. You will be encouraged, but not obligated, to consume and or finish the meal.

3. **Flow-Mediated Dilation (FMD):** The risks associated with the placement of **EKG** electrodes may include redness and/or itching at the electrode sites. The risks associated with forearm occlusion (stopping blood flow to the lower part of your arm) when measuring **FMD** may include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with **ultrasound measurements** may include skin irritation and pressure around the transducer sites. The risks associated with the **Ultrasound lubricant gel** are skin irritation and possible break out of rash. Should a rash occur, the gel will be wiped off, warm compresses will be applied and oral Benedryl will be provided if needed.
4. **Repetitive Blood Draw via Angiocath:** The risks associated with the blood draw via venous catheter h may include infection, irritation and bruising of the skin, pain, discomfort, collapsed vein, multiple puncture sites, and fainting. This risk is minimized by having the catheter inserted in a supine (laying down) or seated position. This risk, although rare, is minimized by using proper sterile technique (such as sterilizing using alcohol swabs). There is a chance of phlebitis which can cause redness, swelling, moderate discomfort, and fever for up to a few days after the catheter is removed. If mild symptoms of phlebitis (swelling (inflammation) of vein caused by a blood clot)develop, warm compresses, alternating Tylenol® and Advil® will be indicated until the symptoms are gone (usually 12-72 hours). If serious symptoms of phlebitis develop (ie. fever or red streaks up the arm) you will be instructed to seek medical care immediately. This risk is minimized by using proper sterile technique.

As in any study, the risks of possible loss of confidentiality exist.

BENEFITS OF TAKING PART IN THE STUDY:

The benefits to participation that are reasonable to expect include information on how a high-fat meal affects your artery health.

COMPENSATION FOR YOUR TIME:

To compensate for the time involved in participating in the study, you will be paid $50/meal for each 5 hour Challenge Meal session for a total of $150. If you are unable to complete all three Challenge Meals, you will be paid $8/hr for the time spent in the lab for the Challenge Meal session. The method of disbursement will be a voucher which asks your name, social security number, address, and ownership status. These data are sent to accounts payable and you will receive a check in the mail.

ALTERNATIVES TO TAKING PART IN THE STUDY:

The only alternative to taking part in this study is to choose not to participate.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Your identity will be held in confidence in reports in which the study may be published. To protect your identity, data about your will be associated with an identification number (rather than your name) and stored in a locked file cabinet in a locked room in the Clinical Exercise Physiology Laboratory. Electronic data will be maintained on a secure drive on the HPER server. You will not be identified in any report of manuscript. Data will be kept in a database and not be discarded. If a subject requests the information to be shared with their primary physician, we will summarize the data and report it to the physician.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the IU Institutional Review Board or its designees, the study sponsor, the AAU/Bell Updyke Research Committee, and (as allowed by law) state or federal agencies, specifically the Office for Human Research Protections (OHRP) who may need to access your research records.

CONTACTS FOR QUESTIONS OR PROBLEMS

For questions about the study or a research-related injury, contact the researchers

Janet P. Wallace, Ph.D. 812 855-6384

Sylvanna Bielko, B.A. 812 855-7556 (lab)

For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to obtain information, or offer input, contact the IU Human Subjects office at (317) 278-3458 (for Indianapolis) or (812) 856-4242 (for Bloomington) or (800) 696-2949.

USE OF SPECIMENS

Blood samples taken in this study will be stored and may be used for different research analyses later.

☐ I give my permission for my blood samples to be used for different research analyses at a later time.

☐ I do not give permission for my blood samples to be used for different research analyses at a later time.

FUTURE RESEARCH

I give my permission to be contacted at later date(s) about later studies in which I may be interested.

☐ I give my permission to be contacted at later date(s) about later studies in which I may be interested.

☐ I do not give my permission to be contacted at later date(s) about later studies in which I may be interested.

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the investigator(s).

SUBJECT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study.

I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Subject's Printed Name:_____

Subject's Signature:_____ **Date**:_____

(must be dated by the subject)

Printed Name of Person Obtaining Consent:_____

Signature of Person Obtaining Consent:_____ **Date**:_____

Form date: July 14, 2011

Appendix F – Recruitment Materials

Volunteers Needed for High-Fat Breakfast & Artery Health Study

Indiana University Clinical Exercise Physiology Laboratory

The purpose of this study is to investigate how meals of differing fat content affects your artery health.

Eligibility Criteria

- Sedentary Healthy Adults 18-40 years old
- No Heart or Pulmonary Disease or Diabetes
- No Gall Bladder Disease
- No lactase intolerance

Testing Procedures

- Fasting Blood Draw at the IU Health Center
- Three High-Fat Meal Challenges
  - 24% fat
  - 52% fat
  - 76% fat
- With measures of
  - Blood biomarkers
  - Artery health

Benefits

- Knowledge of how your arteries respond to a high-fat meal.

Compensation

- $150 for completing all 3 meals ($8/hr)

For more information Contact:

Sylvanna Bielko, B.A.
Clinical Exercise Physiology, HPER 070
Phone: 812.855.7556
E-mail: sbielko@indiana.edu
Study Number:# 0907000527

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Email: sbielko@indiana.edu

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Artery Health Study No # 0907000527
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Artery Health Study No # 0907000527
Tel. 812.855.7556
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(#09-07000527)

e-mail

Thank you for your interest in this study.

The purpose of this study is to see how high-fat meals affect your arteries.

To be eligible for this study you must be 18 to 40 years of age, and must not:

- Be active (>90 min/week)
- Be lactose intolerant
- Have had a heart attack, lung disease, or diabetes
- Have gallbladder disease
- Take medications that dilate your arteries such as nitroglycerin, prostacyclin, or verapamil
- Take medications that lower your cholesterol such as statins
- Take any oral contraceptives (birth control pills)
- Have elevated cholesterol (>240 mg/dL) and/or triglycerides (>200 mg/dL)

The larger commitment to this study would be participation in three breakfast challenge meals; each one being a different high-fat content. The meals will be mixtures of Ensure and whipping cream.

More specifically, each meal challenge testing session will take about five hours. We will take baseline measurements, have the meal, then take measurements again at 2 and 4 hours after the meal. The measurements we will take include an ultrasound picture of your artery and blood, drawing and analyzing blood, and take weight and height measurements. You will also be asked to have a fasting (no food or liquid for 8 hours prior) blood draw taken at the IU Health Center to measure your cholesterol.

Would you like to visit the lab to see the equipment and testing areas and to talk more about the study? Please contact Sylvanna Bielko or Janet Wallace, Ph.D at (812) 855-7556.

Lipemia, oxidative stress and endothelial function: a dose response.

(#0907000527)

Telephone & Face to Face Script

Hello, my name is _____and I am one of the study coordinators of the high-fat meal study at Indiana University.

Thank you for your interest in this study.

The purpose of this study is to see how high-fat meals affect your arteries.

To be eligible for this study you must be 18 to 40 years of age or older, and must not:

- Be active (>90 min/week)
- Be lactose intolerant
- Have had a heart attack, lung disease, or diabetes
- Have gallbladder disease
- Take medications that dilate your arteries such as nitroglycerin, prostacyclin, or verapamil
- Take medications that lower your cholesterol such as statins
- Take any oral contraceptives (birth control pills)
- Have elevated cholesterol (>240 mg/dL) and/or triglycerides (>200 mg/dL)

Do you meet these criteria?

If the answer is NO:

I am sorry; we need to control for these variables. Thank you for your time. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

The larger commitment to this study would be participation in three breakfast challenge meals; each one being a different high-fat content. The meals will be mixtures of Ensure and whipping cream. Are you lactose intolerant?

Are you still interested in participating?

If the answer is NO:

Thank you very much for calling. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

More specifically, each meal will take at least five hours. We will take baseline measurement, have the meal, then take measurements at 2 and 4 hours after the meal. The measurements we will take include an ultrasound picture of your artery and bloods. You will also be asked to have a fasting blood draw taken at the IU Health Center to measure your cholesterol.

Are you still interested in participating?

If the answer is NO:

Thank you very much for calling. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

To participate, you also need to:

- Fast for 12 hours
- Not exercise or perform strenuous physical activity at least 12 hours before each testing session.
- Not have any caffeine at least 8 hours before each testing session.
- Not take any vitamin supplements at least 8 hours before each testing session.
- Not have tobacco products at least 8 hours before each testing session.

Will you be able to follow these instructions?

If the answer is NO:

I am sorry; we need people who will be able to do these things. Thank you for your time. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

We need to schedule an appointment to go over the informed consent during which I will be able to describe all the procedures of the study in detail. This first appointment will take 15-20 minutes.

[Scheduling part]

Thank you very much for your time. I look forward to meeting you on that day.

Have a great day!

Lipemia, oxidative stress and endothelial function: a dose response.

(#0907000527)

Student Recruiting Script

Hello, my name is _____and I am one of the study coordinators of the exercise and high-fat meal study at Indiana University.

We have a study we are conducting in the Clinical Exercise Physiology Lab. We are looking for people who don't exercise more than 90 minutes a week.

The purpose of this study is to see how high-fat meals affect your arteries.

You will first be asked to go to the IU Health Center for a fasting blood draw to measure your cholesterol and triglycerides. After that you will drink three different breakfast meals on three separate mornings. The meals will be mixtures of Ensure and whipping cream. Each meal will be a different fat content; 25, 50 and 75% fat. We will measure your artery response with ultrasound before and at 2 and 4 hours after the meal. You will be asked to stay in the lab for the whole morning. You can study and use our computers during this time. We will also take blood samples at each time to measure the oxidative stress (harmful effects) of the meals.

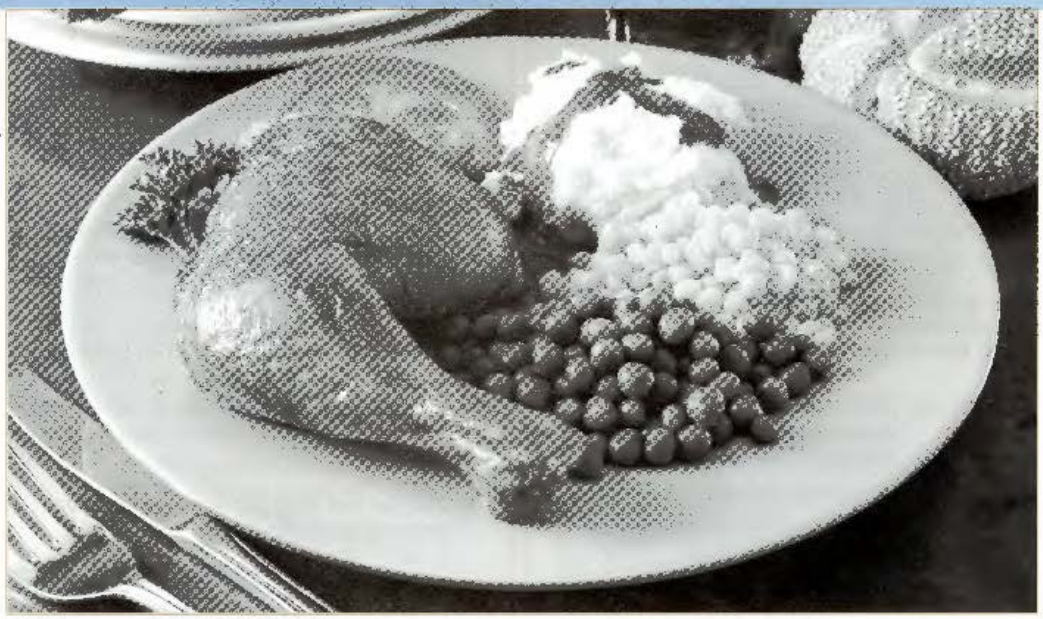
To be eligible for this study you must be 18 years of age or older, and must **not**:

- Be active (>90 min/week)
- Have had a heart attack, lung disease, or diabetes
- Have gallbladder disease
- Take medications that dilate your arteries such as nitroglycerin, prostacyclin, or verapamil
- Take medications that lower your cholesterol such as a statin
- Take any oral contraceptives (birth control pills)
- Have elevated cholesterol (>240 mg/dL) and/or triglycerides (>200 mg/dL)
- Be lactose intolerant

We will leave this flyer in the back of the class. It has our contact information on it. Please consider this study. Parking for the study, blood draws, and breakfasts are free.

Appendix G - Forms

Food Questionnaire



This form asks about your usual food intake during _____ .

Please use **pencil**.

Please **print your name** in this box.

Answer by filling in the correct oval.

● Yes ○ No

Do not make any other marks on the form. Please use a separate piece of paper to make comments.

TODAY'S DATE		
MO	DAY	YEAR

IDENTIFICATION NUMBER

SEX
○ Male
○ Female

GSEL

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Part I: Usual Food Choices

These questions are about the types of foods you ate during _____.

1. Did you eat chicken or turkey?

- ○ Yes → **When you ate chicken or turkey, how often did you eat the skin?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

2. Did you eat beef, pork, ham or lamb?

- ○ Yes → **When you ate beef, pork, ham or lamb, how often did you eat the fat?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

3. Did you eat hamburger or other ground meat?

- ○ Yes → **When you ate hamburger or other ground meat, was it usually...** *Mark one or two.*
  - ○ Regular
  - ○ Lean
  - ○ Extra lean
  - ○ Ground chicken or turkey
  - ○ Don't know
- ○ No

4. Did you drink orange, grapefruit or other fruit juices?

- ○ Yes → **Were any of these vitamins or minerals added (specially fortified) to the juices you drank?** *Mark all that apply.*
  - ○ Extra Vitamin C
  - ○ Vitamin E
  - ○ Calcium
  - ○ None
  - ○ Don't know
- ○ No

5. Did you eat cold cereals?

- ○ Yes → **When you ate cold cereal, what type did you usually eat?** *Mark one or two.*
  - ○ Highly fortified cereals (100% of Daily Values) such as Total®, Smart Start® and Product 19®
  - ○ High fiber or bran cereals such as Raisin Bran® and All Bran®
  - ○ Regular granola (not lowfat)
  - ○ All other cereals such as lowfat granola, Cheerios®, Corn Flakes® and Frosted Flakes®
- ○ No

6. Did you put milk (all types), cream or creamer on cereal?

- ○ Yes → **When you put milk, cream or creamer on cereal, what type did you usually use?** *Mark one or two.*
  - ○ Cream or half and half
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Non-dairy creamer
  - ○ Don't know
- ○ No

7. Did you put milk (all types), cream or creamer in coffee or tea?

- ○ Yes → **When you put milk, cream or creamer in coffee or tea, what type did you usually use?** *Mark one or two.*
  - ○ Cream or half and half
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Non-dairy creamer
  - ○ Don't know
- ○ No

8. Did you drink milk (all types)? *Also include beverages made with milk, such as lattes, cappuccinos, mochas or hot chocolate.*

- ○ Yes → **When you drank milk or beverages made with milk, was it usually...** *Mark one or two.*
  - ○ Whole milk
  - ○ 2% milk
  - ○ 1% milk or buttermilk
  - ○ Nonfat or skim milk
  - ○ Soy milk
  - ○ Don't know
- ○ No

9. Did you use salad dressing?

- ○ Yes → **When you used salad dressing, what type did you usually use?** *Mark one or two.*
  - ○ Regular, including oil and vinegar
  - ○ Low or reduced fat
  - ○ Fat free or nonfat
- ○ No

10. Did you use mayonnaise?

- ○ Yes → **When you used mayonnaise, what type did you usually use?** *Mark one or two.*
  - ○ Regular
  - ○ Low or reduced fat
  - ○ Fat free or nonfat
- ○ No

11. Did you eat cookies or cakes?

- ○ Yes → **When you ate cookies or cakes, how often were they fig bars, SnackWells®, angel food cakes, or other types of low or nonfat cookies or cakes?**
  - ○ Almost always
  - ○ Often
  - ○ Sometimes
  - ○ Rarely
  - ○ Never
- ○ No

12. In your household, what kinds of fat were usually used when cooking, for example to flavor vegetables or fry meat? *Mark up to four.*

- ○ Butter
- ○ Stick margarine
- ○ Tub or liquid margarine
- ○ Lowfat margarine
- ○ Olive oil
- ○ Canola oil
- ○ Other oils such as corn, soybean, peanut and safflower
- ○ Lard, bacon fat or meat drippings
- ○ Didn't use fat or used non-stick spray (such as Pam®)

13. What kinds of fat did you use at the table, for example on breads, vegetables or potatoes? *Mark up to four.*

- ○ Butter
- ○ Stick margarine
- ○ Tub or liquid margarine
- ○ Lowfat margarine
- ○ Olive oil
- ○ Sour cream
- ○ Didn't use fat

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Part II: Usual Food Use

These questions are about foods you ate during _____.

14. Mark the column to show how often, on average, you ate the following foods. Mark your usual serving size as small, medium or large.

- A small serving is about one-half (½) the medium serving size or less.
- A large serving is about one-and-a-half (1½) times the medium serving size or more.

EXAMPLE: This person ate spaghetti with meat sauce every Saturday. They usually ate about 1½ cups.

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Spaghetti, lasagna, and other pasta with tomato with meat sauce	○	○	○	●	○	○	○	○	○	1 cup	○	○	●

CEREALS, BREADS, SNACKS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cold cereals	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Cooked cereals and grits	○	○	○	○	○	○	○	○	○	1cup	○	○	○
Milk on cereals	○	○	○	○	○	○	○	○	○	½ cup	○	○	○
Pancakes, French toast and waffles	○	○	○	○	○	○	○	○	○	2 pieces	○	○	○
Muffins, scones, croissants and biscuits	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
White breads, including bagels, rolls and English muffins	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Dark breads, including dark bagels and rolls	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Cornbread and corn muffins	○	○	○	○	○	○	○	○	○	2 slices or 1 medium	○	○	○
Butter or margarine on breads, cereals, pancakes, etc.	○	○	○	○	○	○	○	○	○	2 pats or 2 teaspoons	○	○	○
Jam, jelly, honey, syrup and sugar (including in coffee, tea and cereal)	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Granola bars and cereal bars such as Nutri-Grain Bars®	○	○	○	○	○	○	○	○	○	1 bar	○	○	○
Sports or meal replacement bars such as Power Bars® and Clif Bars®	○	○	○	○	○	○	○	○	○	1 bar	○	○	○

CEREALS, BREADS, SNACKS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→ Medium serving size	AMOUNT? S	M	L
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day				
Low or nonfat potato chips, tortilla chips, corn chips and pretzels	○	○	○	○	○	○	○	○	○	2 handfuls or 1 sm. bag	○	○	○
Regular potato chips, tortilla chips, corn chips and puffs	○	○	○	○	○	○	○	○	○	2 handfuls or 1 sm. bag	○	○	○
Plain popcorn (no butter) or lowfat microwave popcorn	○	○	○	○	○	○	○	○	○	4 handfuls	○	○	○
Buttered or regular microwave popcorn	○	○	○	○	○	○	○	○	○	4 handfuls	○	○	○
Low or nonfat crackers such as saltines and SnackWells®	○	○	○	○	○	○	○	○	○	6 medium	○	○	○
Regular crackers such as Ritz® and Wheat Thins®	○	○	○	○	○	○	○	○	○	6 medium	○	○	○
Peanut butter, peanuts and other nuts and seeds	○	○	○	○	○	○	○	○	○	2 Tbsp. (spreads) or 1/4 cup (nuts)	○	○	○

MEAT, FISH, EGGS

	HOW OFTEN DID YOU EAT THESE FOODS?									→ Medium serving size	AMOUNT? S	M	L
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day				
Eggs (egg substitute, mark "NEVER")	○	○	○	○	○	○	○	○	○	2 eggs	○	○	○
Bacon and breakfast sausage	○	○	○	○	○	○	○	○	○	3 strips or 2 links	○	○	○
Low or reduced fat hot dogs and sausage	○	○	○	○	○	○	○	○	○	1 hot dog or 2 ounces	○	○	○
Regular hot dogs and sausage such as bratwurst and chorizo	○	○	○	○	○	○	○	○	○	1 hot dog or 2 ounces	○	○	○
Lunch meats such as ham, turkey and lowfat bologna	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
All other lunch meat such as bologna, salami and Spam®	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Canned tuna, tuna salad and tuna casserole	○	○	○	○	○	○	○	○	○	1/2 can tuna or 1 cup casserole	○	○	○
Beef, pork, ham and lamb	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Ground meat, including hamburgers and meatloaf	○	○	○	○	○	○	○	○	○	1 medium patty or 3 ounces	○	○	○
Liver, chicken liver and organ meats	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Fried chicken, including nuggets and tenders	○	○	○	○	○	○	○	○	○	1 large piece or 6 nuggets	○	○	○

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MEAT, FISH, EGGS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Chicken and turkey (roasted, stewed, grilled or broiled)	○	○	○	○	○	○	○	○	○	1 large or 2 small pieces	○	○	○
Fried fish, fish sandwich and fried shellfish (shrimp and oysters)	○	○	○	○	○	○	○	○	○	3 ounces or 1 sandwich	○	○	○
Shellfish, not fried (shrimp, lobster, crab and oysters)	○	○	○	○	○	○	○	○	○	3 ounces or 1 cup	○	○	○
White fish (broiled or baked) such as sole, halibut, snapper and cod	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Dark fish (broiled or baked) such as salmon, mackerel and bluefish	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○

SPAGHETTI, MIXED DISHES, SOUPS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Stew, pot pie, curries and casseroles with meat or chicken	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Chili with meat and beans	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti, lasagna and other pasta with tomato and meat sauce	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti and other pasta with tomato sauce (no meat)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Spaghetti and other pasta with oil, cheese or cream sauce, including macaroni and cheese	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Asian-style (stir-fried) noodles and rice such as chow mein, fried rice and Pad Thai	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Pizza	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Tofu, tempeh and products such as tofu hot dogs, soy burgers and tofu cheese	○	○	○	○	○	○	○	○	○	3 ounces, 1 hot dog or 1 burger	○	○	○
Burritos, tacos, tostadas and quesadillas	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Enchiladas and tamales	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Vegetable, minestrone and tomato soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Cream soups such as chowders, potato and cheese	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

SPAGHETTI, MIXED DISHES, SOUPS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Bean soups such as pea, lentil and black bean	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Miso soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Ramen noodle soup	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Other soups such as chicken noodle	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

DAIRY PRODUCTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cottage cheese and ricotta cheese	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Low or reduced fat cheese, including cheese used in cooking	○	○	○	○	○	○	○	○	○	1 slice or 1/4 cup shredded	○	○	○
All other cheese (American, cheddar or cream), including cheese used in cooking	○	○	○	○	○	○	○	○	○	1 slice, 1/4 cup shredded or 2 Tbsp. cream	○	○	○
Yogurt, all types except frozen	○	○	○	○	○	○	○	○	○	1 cup	○	○	○

VEGETABLES and GRAINS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Mark all vegetables you ate, including in salads, mixed dishes, sandwiches and stir-fries.													
Green salad (lettuce or spinach)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Salad dressing (all types)	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Fresh tomatoes	○	○	○	○	○	○	○	○	○	1 medium or 4 slices	○	○	○
Carrots	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Green peppers and green chilies	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Red peppers and red chilies	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○

VEGETABLES and GRAINS (continued)

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Mark all vegetables you ate, including in salads, mixed dishes, sandwiches and stir-fries.													
Broccoli	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Cauliflower, cabbage and Brussels sprouts	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Green or string beans	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Green peas	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Corn and hominy	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Summer squash and zucchini	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Winter squash such as acorn, butternut and pumpkin	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Yams and sweet potatoes	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Cooked greens such as spinach, mustard greens and collards	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Onions and leeks	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Fresh garlic, including in cooking	○	○	○	○	○	○	○	○	○	1 clove	○	○	○
Avocado and guacamole	○	○	○	○	○	○	○	○	○	1/4 medium or 1/4 cup	○	○	○
French fries, fried potatoes and hash browns	○	○	○	○	○	○	○	○	○	3/4 cup	○	○	○
Potatoes (boiled, baked or mashed)	○	○	○	○	○	○	○	○	○	1 medium or 3/4 cup	○	○	○
Refried beans	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
All other beans (baked, lima or chili without meat)	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Coleslaw	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Potato, macaroni and pasta salads made with mayonnaise or oil	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Rice, noodles and other grains (as a side dish)	○	○	○	○	○	○	○	○	○	3/4 cup	○	○	○
Butter, margarine, sour cream and other fat added to vegetables, potatoes and rice	○	○	○	○	○	○	○	○	○	1 pat or 1 teaspoon	○	○	○

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SAUCES and CONDIMENTS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Cheese sauce and cream sauce	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Meat gravies	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Ketchup	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○
Salsa (as dip or on foods)	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Mayonnaise and mayonnaise-type spreads	○	○	○	○	○	○	○	○	○	2 Tbsp.	○	○	○

FRUITS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Apples, applesauce and pears	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Bananas	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Peaches, nectarines and plums	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Apricots (fresh, canned or dried)	○	○	○	○	○	○	○	○	○	2 medium or 4 halves	○	○	○
Dried fruit (other than apricots) such as raisins and prunes	○	○	○	○	○	○	○	○	○	1/4 cup	○	○	○
Oranges, grapefruit and tangerines (not juice)	○	○	○	○	○	○	○	○	○	1 orange or 1/2 grapefruit	○	○	○
Berries such as strawberries and blueberries	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Cantaloupe, orange melon and mango (in season)	○	○	○	○	○	○	○	○	○	1/4 melon or 1/2 mango	○	○	○
Watermelon and red melon	○	○	○	○	○	○	○	○	○	1 medium slice	○	○	○
Any other fruit such as grapes, fruit cocktail, pineapple and cherries	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○

SWEETS

	HOW OFTEN DID YOU EAT THESE FOODS?									→	AMOUNT?		
	NEVER or less than once per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium serving size	S	M	L
Low or nonfat frozen desserts such as lowfat ice cream, frozen yogurt and sherbet	○	○	○	○	○	○	○	○	○	1 scoop	○	○	○
Ice cream and milkshakes	○	○	○	○	○	○	○	○	○	1 scoop or 1 shake	○	○	○
Pudding, custard and flan	○	○	○	○	○	○	○	○	○	3/4 cup	○	○	○
Doughnuts, pies and pastries	○	○	○	○	○	○	○	○	○	1 medium piece or slice	○	○	○
Cookies and cakes	○	○	○	○	○	○	○	○	○	2 med. cookies or 1 piece of cake	○	○	○
Chocolate, candy bars and toffee	○	○	○	○	○	○	○	○	○	1 regular bar or 2 pieces	○	○	○
Other candy such as Lifesavers®, licorice and jelly beans	○	○	○	○	○	○	○	○	○	4 pieces or 12 jellybeans	○	○	○

PLEASE ANSWER THESE THREE IMPORTANT QUESTIONS!

	NEVER or less than once per week	1-2 per week	3-4 per week	5-6 per week	1 per day	2 per day	3 per day	4 per day	5+ per day
	Note that the frequency headings are different.								
How often did you eat foods that were cooked in fat (pan-fried, sautéed, or deep-fried)? *Count all fat such as margarine, butter, oil or lard.*	○	○	○	○	○	○	○	○	○
How often did you eat a serving of vegetables? *Do not count potatoes, salad or beans.*	○	○	○	○	○	○	○	○	○
How often did you eat a serving of fruit? *Do not count juices.*	○	○	○	○	○	○	○	○	○

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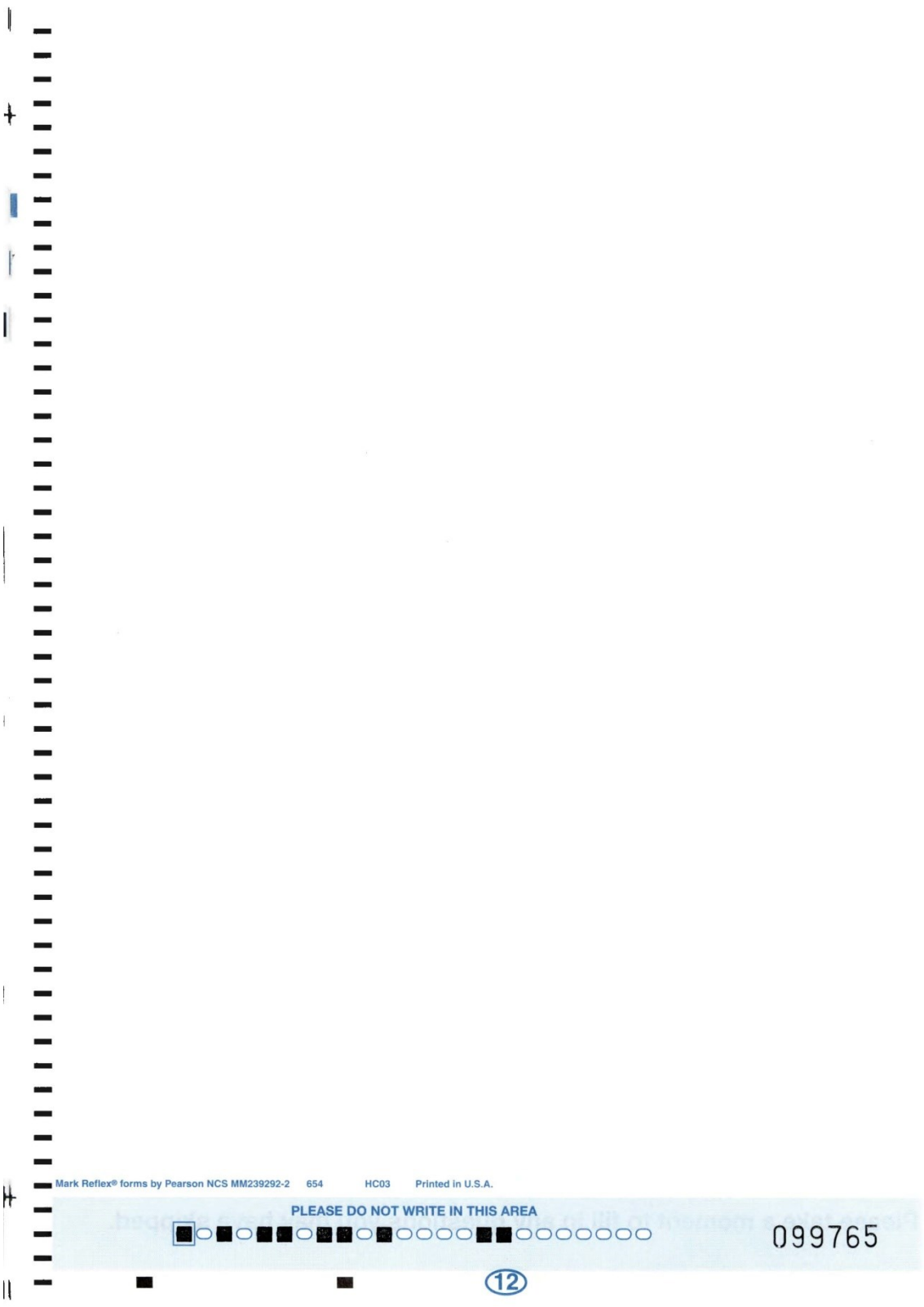
BEVERAGES and ALCOHOL

	HOW OFTEN DID YOU DRINK THESE BEVERAGES?									→	AMOUNT?		
	NEVER or less than once per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day	6+ per day	**Medium serving size**	**S**	**M**	**L**
	Note that the frequency headings are different.												
Milk (all types) as a beverage	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Latte, cappuccino, mocha or hot chocolate	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Coffee (not lattes or mochas)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Tea (all types)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Milk, cream or creamer added to tea and coffee	○	○	○	○	○	○	○	○	○	1 Tbsp.	○	○	○
Tomato juice, V-8® and other vegetable juices	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Orange juice and grapefruit juice	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Other 100% fruit juice such as apple, grape and cranberry	○	○	○	○	○	○	○	○	○	¾ cup	○	○	○
Fruit drinks fortified with Vitamin C such as Hi-C®, Fruitopia® and Kool-Aid®	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Meal replacement drinks and shakes such as Slim•Fast®, Ensure® and Carnation Instant Breakfast®	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Diet soft drinks	○	○	○	○	○	○	○	○	○	12 ounces or 1 can	○	○	○
Regular soft drinks	○	○	○	○	○	○	○	○	○	12 ounces or 1 can	○	○	○
Water (tap, bottled or sparkling)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Beer (all types)	○	○	○	○	○	○	○	○	○	12 ounce can or bottle	○	○	○
Red wine	○	○	○	○	○	○	○	○	○	1 medium glass (6 oz)	○	○	○
White or rosé wine	○	○	○	○	○	○	○	○	○	1 medium glass (6 oz)	○	○	○
Liquor and mixed drinks	○	○	○	○	○	○	○	○	○	1 shot (1½oz) or 1 mixed drink	○	○	○

THANK YOU!

Please take a moment to fill in any questions you may have skipped.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Indiana University

Clinical Exercise Physiology Lab

Medical History/Health Habit Questionnaire

Name_____ Age_____ Birthdate_____
(Please print) Day/Month/Year

Home address_____Zip_____

Work address_____Zip_____

Home phone_____Cell phone_____e-mail_____

1. LIST HOSPITALIZATION HISTORY

Age of Hospitalization	Reason for Hospitalization	Duration of Stay	Comments

2. LIST CHILDHOOD DISEASES

Disease Age

_____

_____

_____

3. LIST ALL MEDICATIONS PRESENTLY TAKING

Medication	Purpose	Dose	How Often

4. FAMILY HISTORY OF HEART DISEASE/STROKE

Indicate immediate family members (parents, siblings, aunts, uncles) who have diagnosed heart disease/stroke and/or who have died from heart disease /stroke

Relationship	Type of Disease	Age at Diagnosis	Age at Death

5. HIGH BLOOD PRESSURE
   a) Have you ever been told you have high blood pressure? _____Yes _____No
   b) If so, when? _____
   c) Was any treatment recommended? _____Yes _____No
   d) If so, what? _____
   e) Are you still undergoing that treatment? _____Yes _____No
   f) If no, when did you stop? _____
   g) List any family members who have had/had high blood pressure

Relationship	Age at Diagnosis

6. DIABETES
   a) Have you ever been told you have diabetes? _____Yes _____No
   b) If so, when? _____
   c) What type of treatment was recommended?
      Diet_____ Exercise_____
      Medication_____ Name of medication_____ Dose_____ Insulin_____
   d) Are you still undergoing treatment? _____Yes _____No
   e) If no, when did you stop? _____
   f) List an family members who have had/had diabetes:

Relationship	**Type of Disease***	**Age at Diagnosis**

* Either Type I (previously known as juvenile diabetes) or Type II (previously known as adult onset diabetes)

7. CHEST DISCOMFORT
   a) Have you ever experienced chest discomfort? _____Yes _____No
   b) If so, when?
      a. Describe the nature of the discomfort
      b. What were you doing at the time?
      c. When does it disappear?
      d. Was medical advice sought? _____Yes _____No
      e. What type of evaluation was performed? _____
      f. What was the result/conclusion of this evaluation? _____
      g. Are you on any medication for chest discomfort? _____Yes _____No
      h. If so, what? _____ How often? _____
      i. How much? _____

8. ARRYTHMIAS
   a) Have you ever experienced skipped heart beats, rapid heart rates or other arrhythmias? _____Yes _____No
   b) If so, when?
   c) What?
   d) Was medical advice sought? _____Yes _____No
   e) What type of evaluation was performed? _____
   f) What was the result/conclusion of this evaluation? _____
   g) Are you on any medication as a result of this evaluation? _____Yes _____No
   h) If so, what? _____How often? _____
   i) How much? _____

9. MUSCULAR/SKELETAL PROBLEMS
   a) Do you have any muscle or skeletal problems? _____Yes _____No
   b) What? _____
   c) Does this limit your ability to exercise? _____Yes _____No
   d) Has medical advice been sought? _____Yes _____No
   e) What was the conclusion of this medical evaluation? _____
   f) Have you ever had any muscle or skeletal problems in the past? _____Yes _____No
   g) What?
   h) Was medical advice sought? _____Yes _____No
   i) What was the conclusion of this medical examination? _____

10. PHYSICAL ACTIVITY
   a) Are you presently engaging in any type of physical activity? _____Yes _____No

Type of Exercise	How Long (min)	How often (days/week)	How Hard (Light-Moderate-Hard)	When Did You Start

b) Have you engaged in any type of physical activity in the past? _____Yes _____No

Type of Exercise	How Long (min)	How often (days/week)	How Hard (Light-Moderate-Hard)	When Did You Start	When Did You Start	When Did You Quit	Why

c) Occupation_____ Years at present work status
d) If retired, what was your occupation?
e) Do you consider your day: _____Sedentary? _____Moderately active? _____Heavy work?
f) How many hours do you spend sitting each day? _____

11. How many hours do you sleep a night? _____ Soundness of sleep:_____

12. STRESS
    a) Do you consider your day stressful? _____Yes _____No
    b) What is the nature of your stress? _____
    _____
    c) How do you handle your stress? _____
    _____

13. Which meals do you eat?

	Daily	Occasionally	Never
Breakfast			
Early morning snack			
Lunch			
Afternoon snack			
Dinner			
Bedtime snack			

14. WEIGHT
    a) Do you consider yourself overweight? _____Yes _____No
    b) How long have you been overweight? _____
    c) How many pounds would you like to lose? _____

15. SMOKING HISTORY
    a) Do you smoke? _____Yes _____No
    b) How much? _____
    c) Have you ever smoked in the past? _____Yes _____No
    d) What did you smoke? _____
    e) How many years? _____
    f) How much? _____
    g) When did you stop? _____
    h) Why? _____

16. ALCOHOL CONSUMPTION
    a) Do you drink alcohol? _____Yes _____No
    b) What kind? _____
    c) How often? _____
    d) Did you ever use alcohol in the past? _____Yes _____No
    e) What? _____
    f) How much? _____ How often? _____
    g) How many years? _____ When did you start? _____

17. List any known allergies:_____
_____

Any additional pertinent information_____
_____
_____
_____

Signature_____ Date_____

OFFICE USE ONLY

*NOTES:*_____
_____
_____
_____
_____
_____
_____
_____

Signature_____ Date_____

BLOOD DRAW INSTRUCTIONS

The Clinical Exercise Physiology lab for the study entitled "Lipemia, oxidative stress, and endothelial function: a dose response" requires that all individuals scheduled for testing have their blood drawn. In order to us to obtain the blood chemistry results prior to your scheduled testing date, the blood draw needs to be done before _____, _____.

Please go to the Indiana University Health Center at the corner of 10th Street and Jordan Avenue for this procedure. The hours are Monday & Thursday from 8 am to 5:30 pm and Tuesday, Wednesday, & Friday from 8 am to 4:30 pm. They will have your name and will be expecting you. From the parking lot entrance, the blood lab is the last room on the right side of the hall (Room 208). The blood draw will take 10-15 minutes. Because you need to fast before having your blood drawn, **DO NOT EAT 12 HOURS BEFORE THE PROCEDURE**. Thank you.

Date: _____

Participant Name: _____

Participant DOB: _____

Participant ID: _____

The above person needs to have a lipid panel. Fax completed results to 855-8179.

CEP Lab: This information needs to be emailed to Donna Dayton at ddayton@indiana.edu.

Clinical Exercise Physiology Lab

Appointment Reminder for the study

Lipemia, oxidative stress, and endothelial dysfunction: a dose response

Name:_____

You will need to come into the lab for the three high-fat breakfast meals on three separate days. Your three meals testing will take place on:

Meal 1: _____ at _____

date time

Meal 2: _____ at _____

date time

Meal 3: _____ at _____

date time

In order to participate in the high-fat meals you must:

- Not exercise for at least 12 hrs before each meal
- Not have any caffeine at least 8 hrs before each meal
- Not have any Vitamin supplementation for at least 8 hrs before each meal
- Not have any tobacco products at least 8 hrs before each meal
- Be fasting for the past 12 hrs; drink plain water only
- If you are on any medications that dilate your arteries and/or cannot be tested within the preparation criteria listed above you will be excluded from the study

Please make sure to wear a short sleeve shirt for each of the meals. You need your arms to be accessible for both the FMD and blood draws.

If you have any questions or concerns, please contact Sylvanna Bielko via email at sbielko@indiana.edu or call (812) 855-7556 (lab) or (260) 437-1379 (Sylvanna's personal cell)

Thanks and see you soon!